Influence of Nicotine in Osseointegration of Dental Implants

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Abstract

The use of endosseous implants as a foundation for the prosthetic replacement of missing teeth has become widespread in dental practice. Owing to the remarkable success of dental implants, there has been growing interest in identifying the factors associated with implant failure. Given the well-documented deleterious effect of smoking on wound healing after tooth extraction and its association with poor bone quality and periodontal disease, a negative effect of tobacco use on implant success is to be expected.

Due to that, this work aims to establish a relationship between a major compound of tobacco particulate smoke, nicotine, and implant osseointegration, including the incidence of complications related to these procedure and the long-term survival and success rates of dental implants among smokers based on relevant literature. Furthermore, this work will search for the effects of nicotine at the cellular level of the bone, in order to understand if it is the unique, or, at least, the main responsible for the deleterious effects of smoking on the outcome of dental implants osseointegration.

Resumo

A utilização de implantes dentários como suporte para a substituição protética de dentes ausentes tornou-se uma prática comum na Medicina Dentária. Devido ao enorme sucesso dos implantes dentários, tem havido um interesse crescente em identificar os factores associados ao insucesso dos mesmos. Devido ao conhecido efeito nocivo do tabaco na cicatrização após extracções dentárias, e a sua associação com osso de baixa qualidade e doença periodontal, é de esperar um efeito negativo no sucesso da colocação de implanentes dentários.

Deste modo, este trabalho tem como objectivo estabelecer uma relação entre o principal componente do fumo do tabaco, a nicotina, e a osseointegração de implantes dentários, incluindo a incidência de complicações relacionadas com este procedimento e a sobrevivência dos mesmos a longo prazo em fumadores, tudo isto baseado em literatura relevante sobre este tema. Além disso, este trabalho vai procurar esclarecer os efeitos da nicotina ao nível celular do tecido ósseo, de forma a perceber se esta é única, ou, pelo menos, a principal responsável pelos efeitos nocivos do tabaco no sucesso da colocação de implantes dentários.
Key words

Endosseous implants, Nicotine, Osseointegration, Osteoblasts, Osteoclasts, Smoking.

Introduction

Cigarette smoking is still considered a common habit these days. Despite this fact, many actions are being taken in order to stop or prevent more people from smoking. This actions are partially fueled by the recognition of the increasing number of diseases with which smoking has been directly or indirectly associated, and also because smoking is an avoidable cause or morbidity and mortality. The adverse effects on the cardiovascular system are well known, and these effects are also implicated in the etiology of many cancers. Nearly 4000 different gases and chemicals are released during cigarette smoking, including nitrogen, carbon monoxide, carbon dioxide, ammonia, hydrogen cyanide, benzene, nicotine, nornicotine, anatabine, and anabasine[1]. Nicotine, considered the major and the addictive component of the particulate phase of tobacco smoke, has been implicated in the pathogenesis of numerous diseases that affect different tissues of the organism. One of them is bone tissue, where nicotine seems to play a significant role in the adverse effects of smoking affecting the normal bone metabolic activities, as well as the healing process of damaged bone[1, 2].

In smokers, increased plaque accumulation, higher incidence of gingivitis and periodontitis, higher rate of tooth loss, and increased resorption of the alveolar ridge can be found in the oral cavity. Also, cigarette smoking may adversely affect wound healing, and so put in risk the success of bone grafting and dental implantation[1].

The use of endosseous implants has increased over the past decade in many edentulous situations, and as a chirurgical act that it is, every step must be done carefully, in an attempt to minimize the odds of failure. An endosseous implant needs a correct osseointegration to the bone in order to be considered successful. Osseointegration of dental implants, essential for long term-clinical success, involves the direct anchorage of the implant by the formation of new bone (without the growth of fibrous tissue) at the bone-implant interface, and represents a dynamic process both during its establishment and its maintenance[2].
The implant osseointegration and maintenance require the recruitment of osteoblast precursor cells, their anchorage, attachment, adhesion, spreading, proliferation, and differentiation into secretory osteoblasts with the production of a calcified extracellular matrix at the implant surface[2-4]. These cellular events are highly sensitive to the local microenvironment and both systemic and local effects of nicotine/ tobacco components have been associated with negative effects osteoblastic activities. Although the reported success rate of titanium implants is high, failure does occur, and smoking is one of the factors often associated to implant failure[1, 2, 5, 6].

In that context, Haas and Haimböck[7], in a retrospective study, concluded that local factors can have detrimental effects around successfully integrated maxillary implants, with a significantly higher bleeding index, higher mean peri-implant pocket depth, more frequent periimplant inflammation, and radiographically higher mesial and distal bone loss. Lindquist et al. [8] compared marginal bone loss (MBL) around osseointegrated dental implants among smokers and nonsmokers. They concluded that in smokers who also had poor oral hygiene, MBL was nearly 3 times as high as that in nonsmokers.

Experimental studies also showed that the oral mucosa can be highly permeable to nicotine and the junctional peri-implant epithelium, although structurally and functionally identical to junctional epithelium around natural teeth, presents a high level of permeability to exogenous substances. Considering the high levels of nicotine attained in the crevicular fluid and saliva of tobacco users and the high lipophilic and diffusible character of this molecule, it is very likely that in those individuals there might be significant levels of nicotine at the bone/implant interface[2].

Previous studies evaluated the influence of nicotine at the bone cellular level, namely at the equilibrium between bone formation and resorption [9, 10]. Henemyre et al.[11] found that nicotine appears to stimulate osteoclast differentiation and resorption of calcium phosphate, the major component of bone, which is in line with the increased speed of periodontal bone loss and refractory disease incidence in smokers [11]. In addition, Ma, L. et al. [12] observed that nicotine directly inhibits the proliferation, and the expression of osteogenic and angiogenic mediators by osteoblasts. It was also observed that nicotine can interfere with implants osseointegration, due to the fact that it might inhibit the bone matrix-related genes expression that are crucial for the success of that process [13]. Another important cell at the implant-bone interface, the fibroblast, may also be influenced by nicotine as well. Giannopoulou et al. [14]. observed that nicotine inhibited the proliferation, attachment, ALP
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(alkaline phosphatase) production and migration of human periodontal ligament fibroblasts, suggesting that it may impair the host defense system against the progression of periodontitis.

Taken together, the purpose of this article is to describe the relationship between cigarette smoking/nicotine and implant correct osseointegration, including the incidence of complications related to these procedure, and long term survival and success rates of dental implants among smokers and nonsmokers. The discussion is based on relevant literature and results of recent studies. The facts presented will assist dental health professionals in treatment planning decisions and provide them with important information to share with patients who use tobacco products.

Materials and Methods

Relevant clinical studies published in English between 1996 and 2011 were reviewed. The articles were located through Pubmed® and, manually, through the references of peer-reviewed literature.

Bone Tissue

Bone is a specialized connective tissue hardened by mineralization on its extracellular matrix with calcium phosphate in the form hydroxyapatite ([Ca$_3$(PO$_4$)$_2$]Ca(OH)$_2$). Bone has well recognized mechanical functions: it provides rigidity and shape, protection and support for body structures, and aids locomotion. Contrary to popular belief, bone is in fact a highly dynamic structure undergoing constant remodeling. This bone turnover allows the bone to repair itself, for example after fracture, and to adapt to the forces placed on it[3, 9].

In general each bone has an outer layer of cortical bone overlying trabecular bone and the medullary cavity. The cortical bone has an outer membrane called the periosteum and presents two layers: an outer fibrous layer and an inner one, which has osteogenic potential and lays down new bone allowing the bone to enlarge, a process known as periosteal apposition. The inner surface of the cortex has another lining called the endosteum. Bone tends to undergo resorption from the endosteal surface[9].
Bone has three distinct cell types: the osteoblasts, or bone-forming cells and the osteoclasts, or bone-resorbing cells, whose functions are intimately linked, and the osteocytes, which are osteoblasts entrapped within lacunae inside bone tissue. In order to balance bone formation and resorption in healthy individuals, osteoblasts secrete factors that regulate the differentiation of osteoclasts and osteocytes secrete factors regulating the activity of both osteoblasts and osteoclasts. Bone is constantly being resorbed by osteoclasts and then replaced by osteoblasts in a process called bone remodelling. Resorption is much faster than formation: an area of bone can be resorbed in 2-3 weeks but it takes at least three months to rebuild it. Both the periosteum and endosteum contain osteoblasts and osteoclasts and their progenitors [9, 10].

**Osteoblasts**

Osteoblasts are responsible for the deposition of bone matrix and for osteoclast regulation. They are specialized mesenchymal cells that undergo a process of maturation where genes like core-binding factor a1 (Cbfa1), osterix (Osx) and the Wnt/b-catenin pathway play a very important role[10]. As they differentiate they acquire the ability to secrete bone matrix. Ultimately, some osteoblasts become trapped in their own bone matrix giving rise to osteocytes which, gradually, stop secreting osteoid [10]. The cellular events involved in bone formation are chemotaxis and proliferation of osteoblast precursors and their differentiation to the mature bone matrix-producer osteoblast. All these events are controlled by both systemic hormones and local growth factors[15].

As mentioned before, these cells have also a role in the regulation of bone resorption through the expression of several growth factors, especially the receptor activator of nuclear factor-kB (RANK) ligand (RANKL), that binds to its receptor, RANK, on the surface of pre-osteoclast cells, inducing their fusion and differentiation[10]. On the other hand, osteoblasts secrete a soluble decoy receptor for RANKL, osteoprotegerin (OPG) that blocks RANK/RANKL interaction and, thus, prevents osteoclast genesis. Therefore, the balance between RANKL and OPG is a main determinant of the formation and activity of osteoclasts[10].
Osteoclasts

Osteoclasts are multinucleated cells of monocyte/macrophage lineage that are formed to carry out the unique function of resorbing bone matrix. When attached to bone, differentiated osteoclasts form a closed compartment beneath them, in which the mineral phase of bone, mainly hydroxyapatite, is resorbed by means of acid, and the organic phase, mostly collagen I, is solubilised by proteolytic enzymes, such as lysosomal cysteine proteinases and matrix metallo proteinases. This concerted activity leaves behind resorption pits and trails named Howship’s lacunae. In the second phase of the bone-remodeling process, bone lining cells clean the resorption pits of bone matrix leftovers such as collagen fragments and deposit a thin layer of fibrillar collagen type I. Thereafter, bone tissue is rebuilt by osteoblasts. Through regulation of the local concentration of calcium and phosphate, the formation of hydroxyapatite is promoted[16].

Osteocytes

Osteocytes are the most abundant cells in bone; these cells communicate with each other and with the surrounding medium through extensions of their plasma membrane. Therefore, osteocytes are thought to act as mechanosensors, instructing osteoclasts where and when to resorb bone and osteoblasts where and when to form it[9].

Endosseous implants

Currently the use of osseointegrated implants to treat partially or completely the edentulous arch is considered reliable and predictable, with a success rate of 98% or higher[17]. Installation of implants in the alveolar process elicits a sequence of healing events including necrosis and subsequent resorption of traumatized bone around the titanium body concomitant with new bone formation. While the implant displays initial mechanical stability due to contact and friction between the implant surface and the severed bone, the long-term maintenance of implant stability calls for a biologic attachment between the foreign body and the surrounding tissue[4]. The common clinical end point of this process is measured by a
lack of signs and symptoms of aggressive chronic inflammation, a lack of mobility and a radiographic assessment of bone adapted to the interface[18].

The causes and mechanisms of early implant failure are unclear. Different studies have found a variety of statistically significant factors associated with it, such as: age and sex, systemic diseases, smoking, type of edentulism, maxillary implant location, quantity and quality of bone, implant length and diameter, and immunological and genetic factors[5, 19]. The shape of the implant surface and the type of bone where it is placed seem to be major factors in the success of the implant. Implants with rough surface seem to accomplish better results, and those placed in the mandible have higher survival rates than the ones placed in the maxilla. [17, 20, 21]. Also, bone type 4 (thin layer of cortical bone that surrounds low density trabecular bone) is associated with a lower success rate of long-term implantation [17, 21].

Cancellous bone has a very high surface area, which is contiguous with the bone marrow compartment. Since marrow contains not only mesenchymal progenitor cells that can give rise to osteoblasts, but also a rich vasculature that can supply both the circulating mononuclear precursors to osteoclasts (needed for remodeling) and the endothelial population needed for angiogenesis, it is not surprising that trabecular bone can be remodelled far more quickly than cortical bone. From this perspective, therefore, trabecular bone represents a biologically tissue, ideally evolved for rapid (peri-implant) bone healing, when compared to the slowly remodeling healing pattern typical of cortical bone[3].

The peri-implant bone healing, which results in contact osteogenesis (bone growth on the implant surface), can be phenomenologically subdivided into three distinct phases that can be addressed experimentally. The first, osteoconduction, relies on the migration of differentiating osteogenic cells to the implant surface, through a temporary connective tissue scaffold. Anchorage of this scaffold to the implant is a function of implant surface design. The second, de novo bone formation, results in a mineralized interfacial matrix, equivalent to that seen in cement lines in natural bone tissue, being laid down on the implant surface. Implant surface topography will determine if the interfacial bone formed is bonded to the implant. A third tissue response, bone remodeling, will also, at discrete sites, create a bone-implant interface comprising de novo bone formation[22].

Dental implant failures were divided into early and late failures. Early failures were defined as those occurring between first- and second-stage surgery. Late failures were defined as those occurring after second-stage surgery. Early failure of an implant results from an inability to establish intimate bone-to-implant contact. This means that bone healing after
implant insertion is impaired or jeopardized. The mechanisms that normally lead to wound healing by means of bone apposition fail, and instead, a fibrous scar tissue is formed around the implant. Late failure of an implant has been associated both with peri-implantitis and with plaque-related gingivitis and/or occlusal overloading[23]

**Process of bone healing around Endosseous implants**

Around endosseous implants, osteoblasts may lay down bone on the old bone surface or on the implant surface itself. This distinction was explored by Osborn and Newesley, who described the two phenomena, distance and contact osteogenesis, by which bone can become juxtaposed to an implant surface[3].

In distance osteogenesis, new bone is formed on the surfaces of old bone in the peri-implant site. The bone surfaces provide a population of osteogenic cells that lay down a new matrix that encroaches on the implant. An essential observation here is that new bone is not forming on the implant, but the latter does become surrounded by bone. Thus, in these circumstances, the implant surface will always be partially obscured from bone by intervening cells. Distance osteogenesis can be expected in cortical bone healing since vascular disruption of the cortex caused during implant site preparation is known to lead to death of the peri-implant cortical bone and its subsequent slow remodeling by osteoclast invasion from the underlying medullary compartment[3].

In contrast, in the process of contact osteogenesis, new bone forms first on the implant surface. Since, by definition, no bone is present on the surface of the implant upon implantation, that region has to become colonized by bone cells before bone matrix formation can begin. This is also what happens at bone remodeling sites where a resorption surface of old bone is populated with osteogenic cells before new bone can be laid down[3].

The common factor linking normal tunneling remodeling and contact osteogenesis is that bone is formed for the first time at the appropriate site by differentiating osteogenic cells. This is called *de novo* bone formation. Clearly an essential prerequisite of *de novo* bone formation is that bone cells must first get to either the old bone or implant surface respectively, before extracellular matrix synthesis is initiated. The result of it is that the implant-bone interface, in an identical fashion to remodeling bone surfaces, is occupied by a cement line matrix[3].
The term “osteoconduction” is used to combine the important early events that will position the osteogenic cells on the surface of the implant where they can produce bone matrix. The de novo formation of bone itself can therefore be considered as a separate and distinct phenomenon which, in time, will be followed by the remodeling of the peri-implant bone. The combination of osteoconduction and bone formation will result in contact osteogenesis[3].

The migration of osteogenic cells in peri-implant healing will occur through the transient three-dimensional biological matrix formed as a product of the coagulation cascade— the fibrin of the blood clot—and may be both potentiated and directed, either directly or indirectly through knock-on stimulatory events involving leukocytes, by the release of cytokines, growth factors, and microparticles from platelets activated by contact with the implant surface[3].

Thus, the ability of an implant surface to retain fibrin during the wound contraction phase of healing is critical in determining if the migrating cells will reach the former. The implant surface design will play an important role in this fibrin retention. Thus, it has a profound influence on osteoconduction not only by modulating the levels of platelet activation, but also by maintaining the anchorage of the temporary scaffold through which these cells reach the implant surface[3].

Finally, when the osteogenic cells reach the implant surface, they can initiate bone matrix formation, and after this, the de novo bone formation. This process can be arbitrarily subdivided into a four-stage process, which has been confirmed by both in vitro and in vivo experiments. Briefly, differentiating osteogenic cells initially secrete a collagen-free organic matrix that provides nucleation sites for calcium phosphate mineralization. There are two non-collagenous bone proteins, osteopontin and bone sialoprotein, and two proteoglycans important for this initial organic phase, but no collagen. The lack of collagen in this matrix concurs with the original observations of Weidenreich. Calcium phosphate nucleation is followed by crystal growth and the initiation of collagen fiber assembly. Finally, calcification of the collagen compartment will occur. Thus, in this process of de novo bone formation comprising the second key element of contact osteogenesis, the collagen compartment of bone will be separated from the underlying substratum by a collagen-free calcified tissue layer containing noncollagenous bone proteins[3].

Therefore, distance osteogenesis will result in bone approximating the implant surface while contact osteogenesis results in bone apposition to the implant surface. Although it is
inevitable that both distance and contact osteogenesis occurs in every endosseous healing site, the biological significance of these different healing reactions is of critical importance in both attempting to unravel the role of implant design in endosseous integration and elucidating the differences in structure and composition of the bone/implant interface[3].

**Nicotine**

Nicotine (1-methyl-2-(3-pyridyl)pyrrolidine) is a natural ingredient acting as a botanical insecticide in tobacco leaves. It is the principal tobacco alkaloid, representing about 1.5% by weight in commercial cigarette tobacco and comprising about 95% of the total alkaloid content. Oral snuff and pipe tobacco contain concentrations of nicotine similar to cigarette tobacco, whereas cigar and chewing tobacco have only about half that nicotine concentration. An average tobacco rod contains 10–14 mg of nicotine and on average about 1–1.5 mg of nicotine is absorbed systemically during smoking. In most tobacco strains, nornicotine, anatabine and anabasine are the following most abundant alkaloids. This order of abundance is the same in cigarette tobacco and oral snuff, chewing, pipe, and cigar tobacco[24, 25].

Absorption of nicotine across biological membranes depends on pH. Nicotine is a weak base with a $pK_a$ of 8.0. In its ionized state, such as in acidic environments, nicotine does not rapidly cross membranes. The pH of smoke from flue-cured tobaccos, found in most cigarettes, is acidic (pH 5.5–6.0). At this pH, nicotine is primarily ionized. As a consequence, there is little buccal absorption of nicotine from flue-cured tobacco smoke, even when it is held in the mouth. Smoke from air-cured tobaccos, the predominant tobacco used in pipes, cigars, and some European cigarettes, is more alkaline (pH 6.5 or higher) and, thus, considerable amounts of nicotine is unionized. Smoke from these products is well absorbed through the mouth[24, 25].

Nicotine is extensively metabolized by the liver. Six primary metabolites of nicotine have been identified. Quantitatively, the most important metabolite of nicotine in most mammalian species is its lactam derivative, cotinine. In humans, about 70–80% of nicotine is converted to cotinine[24].

Nicotine accumulates markedly in gastric juice and saliva. Gastric juice/plasma and saliva/plasma concentration ratios are 61 and 11 with transdermal nicotine administration, and 53 and 87 with smoking, respectively[24].
Influence of smoking in the osseointegration of dental implants

There are in the literature many studies regarding the success rate of implant therapies in smokers and nonsmokers patients. Aglietta et al. [26] observed that 75% of implants in smoking periodontally compromised patients yielded at least one site with marginal bone loss ≥3mm, whereas in smoking periodontally healthy patients, the corresponding proportion was 20%. On the other hand, outcomes in nonsmokers showed that 55% of implants in smoking periodontally compromised patients and 12.5% of implants in smoking periodontally healthy patients displayed at least one site with marginal bone loss 3mm after 10 years.

De Luca et al. [27] observed that the overall implant failure rate was 7.72%, and that patients who were smokers at the time of implant surgery had a significantly higher rate (23.08%) than nonsmokers (13.33%).

Also, Cavalcanti et al. [28] suggested that when considering all implant failures up to 5 years after loading, significantly more failures (5.5%) occurred in smokers compared with non-smokers (2.9%) (OR 1.72; 95% CI 1.20 to 2.50; P = 0.003). Five years after loading, smokers experienced almost twice as many implant failures compared with non-smokers. Non-statistically significant trends in favour of non-smokers were also observed for early implant failures and prosthesis failures.

Sánchez-Pérez et al. [6] observed, that the use of tobacco involves a 15.8% risk of implant failure, with a 13.1 odds ratio. In light or moderate smokers smokers tobacco use involves a 10.1% relative risk of implant loss, whereas the consumption of >20 cigarettes per day (heavy smokers) increases this risk to 30.8%.

Stijn Vervaeke et al. [29] performed a study where they have applied one-thousand one-hundred and six implants in 300 patients (186 females; 114 males) with a mean follow-up of 31 months (SD 7.15; range 24–58). Nineteen implants in 17 patients failed, resulting in an overall survival rate of 98.3% at the implant level and 94.6% at the patient level. After a follow-up period of 2 years, the cumulative survival rates were 96.7% and 99.1% with the patient and implant as the statistical unit, respectively. Implant survival was significantly
higher for nonsmokers compared with smokers (implant level $P=0.025$; patient level $P=0.017$). The overall mean bone loss was 0.34 mm (%1076; SD 0.65; range 0–7.1). Smokers lost significantly more bone compared with nonsmokers in the maxilla (0.74 mm; SD 1.07 vs. 0.33 mm; SD 0.65; $P=0.001$), but not in the mandible (0.25 mm; SD 0.65 vs. 0.22 mm; SD 0.5; $P=0.298$).

In addition to this studies, other literature reviews and meta-analysis also show an association between smoking and endosseous implant failure[30-32].

Although there are evidences that highlight the deleterious effects of smoking in endosseous implants therapeutics, there are also studies who concluded that tobacco use cannot be considered a risk for early implant failure[33].

Other authors defend that smoking is well known to have an impact on early failures rate, which may be explained mainly by the effect of smoking on the wound healing process, in early stage of osseointegration, while other factors are more predominant for the late failures [34].

In order to improve the outcome of endosseous implants in smoking patients, some authors have investigated the effects of different types of implant surfaces in such individuals. D’Avila et al.[35] observed that the sandblasted acid-etched surface presented better results than the machined surface after a short healing time in smokers. Other studies also suggest that microstructured implant surfaces (roughened) seem to have a positive influence on marginal bone level around implants in smokers[32, 36].

Nevertheless, as outlined in many of the previously mentioned reports,, it is decisive an implementation of a smoking-cessation protocol, which yields positive outcomes in improving the success rates of dental implants in smokers[26, 29, 31, 33, 37].

Taken together, although cigarette smoking should not be an absolute contraindication for implant therapy, smokers should be informed that they are at a greater risk of implant failure if they smoke during the initial healing phase following implant insertion or if they have a significant smoking history[27].
Effects of nicotine/smoking in the osseointegration of implants

Among the different molecules liberated on tobacco smoke, nicotine is considered the main responsible for the deleterious effect of smoking on implant success rates.

Nicotine and its metabolite cotinine have been found in the saliva and gingival crevicular fluid of smokers[38]. The direct cutaneous vasoconstrictive action of nicotine, the increased levels of fibrinogen, hemoglobin and blood viscosity, excessive levels of carboxyhemoglobin in blood, compromised polymorphonuclear neutrophil leukocyte function, as well as increased platelet adhesiveness have all been hypothesized to be important mechanisms by which smoking compromises wound healing[1, 23, 31, 39]. Decreased polymorphonuclear neutrophil function is also supposed to lower resistance to infections[23]. Also, nicotine, as a major component and most pharmacologically active agent in tobaccos is likely to be a significant contributing factor for the exacerbation of periodontal diseases[25].

Curiously, César-Neto et al.[40] observed that in cortical bone, cigarette smoke presented a significant negative influence on BIC (bone-to-implant contact) and BA (bone area). In contrast, the administration of nicotine did not influence those parameters. In cancellous bone, cigarette smoke inhalation also resulted in a decreased percentage of BIC compared to the control group. In addition, the BA was significantly decreased in groups 2 and 3 (goup2: intermittent cigarette smoke inhalation, n = 15; group 3: subcutaneous administration of nicotine (3 mg/kg) twice daily) when compared to controls. So, they concluded that the negative impact of smoking on implant outcomes may be related to more than one molecule present in the cigarette smoke and nicotine seems to partially contribute to the observed effects, especially in cancellous bone.

Gotfredsen et al.[41] analyzed the effect of a longstanding (6 months) nicotine exposure on osseointegration of titanium implants. They used cotinine, since it has a much longer half time than nicotine, and there is less fluctuation in cotinine concentration, than with nicotine. The tested plasma cotinine levels corresponded to those measured in patients who smoke one to two packs of cigarettes a day. The histometric analysis revealed no significant difference in bone-to-implant contact or bone-density within the bone bed sites between the animals exposed to nicotine and the controls.
Moreover, Nociti et al. [38] did not observed any statistical significant differences between nicotine-exposed and control subgroups (P = 0.74) regarding it’s effects on bone densities. Their work also provided an insight into the effects of nicotine in its common use for smoking cessation (usually transdermal patches of nicotine), and nicotine administration which may not influence bone healing in bone-to-implant contact and bone area. They concluded that daily nicotine administration may not significantly influence bone density around titanium implants.

Balatsouka et al.[42] performed an experimental study in rabbits where test group was exposed to nicotine tartrate for 8 weeks and the control group was exposed to placebo. Implants were placed after 4 weeks (right tibia) and after 6 weeks (left tibia) in the proximal and distal bone bed. Subsequently, 2 and 4 week healing groups were created. The mean BIC in the test group was 46.2 ± 9.3% and 51.3 ± 6.7% for the 2 and 4 weeks of bone healing, respectively. The mean BIC in the control group was 44.2 ± 7.8% and 57.6 ± 6.9% for the 2 and 4 weeks, respectively. A significant difference was found between 2 and 4 weeks in the control group, but not in the test group. Nevertheless, significant difference were found between the test and the control groups, regarding the histomorphometric parameters assessed

Furthermore, in another similar study [43], the same authors observed that nicotine had no effect on early signs of osseointegration assessed by histomorphometry and on implant stability or anchorage.

Soares et al. [39] evaluated the harmful effects of alcohol and nicotine, when consumed simultaneously, on bone mechanical resistance and bone neoformation close to implants. Their results showed that alcohol dealt a greater harm in relation to nicotine, demonstrated by the lower bone volume formed in that group. On the other hand, the combination of the two drugs caused an increased negative effect on the volume of formed bone.

Considering all this, and despite the fact that nicotine is one of the most active components of tobacco, the presented results show some contradictory effects, which might reveal that nicotine may not be the main responsible for the deleterious effect of smoking on the osseointegration of dental implants, or, at least, those effects are not caused only by the action of nicotine. Indeed, many author have suggested that other tobacco components, individually or even in association with nicotine, can be the real cause of the lower success rate of endosseous implants in smokers[38, 40, 41].
Nicotine and Bone Cells

Nicotine is known to affect the behavior of cells important for normal bone metabolic activities. In that context, Giannopoulou et al. [14] observed that nicotine inhibited the proliferation, attachment, ALP production and migration of human periodontal ligament fibroblasts, suggesting that it may impair the host defense system against the progression of periodontitis, and peri-implantitis.

Gullihorn et al. [44] observed that nicotine and smoke condensate differentially affect metabolism of cultured osteoblastlike cells. Nicotine at levels normally achieved in the circulation of smokers promoted metabolic cellular activity in contrast to the inhibitory effect of smoke condensate containing an equivalent amount of nicotine. The reduction of nicotine activity by addition of a nicotine receptor antagonist, Mecamylamine, supported the specificity of nicotine action.

Katono et al. [45] results suggested that nicotine stimulates turnover of the bone matrix by increasing production of MMP-1, 2, 3, and 13 and tPA in osteoblasts, which tips the balance between bone production and resorption toward the latter process, promoting the resorption of alveolar bone in the mouth of smokers.

Rothem et al. [46] observed that nicotine acts on osteoblastic cells through binding to nAChR (nicotinic acetylcholine receptor), leading to a suppressesion of osteogenesis. Moreover, they reported a biphasic effect of nicotine on MG63 osteoblast-like cell proliferation and expression of osteocalcin, type I collagen, and ALP genes: toxic and antiproliferative effects at a high concentration of nicotine and stimulatory effects at low levels of nicotine.

In Yamano et al. [13] study in rats, systemic exposure to nicotine did not affect rat bone development, but bone wound healing around implants was affected. The expression levels of OPN, Col-II, BMP-2, BSP, and CBFa-1 at 4 weeks were significantly down-regulated in the nicotine-exposed group compared with the control, though BMP-2, BSP, and Col-2 genes at 2 weeks were significantly up-regulated in the same group. Their findings suggest that nicotine might inhibit the bone matrix-related gene expressions required for wound healing and thereby diminish implant osseointegration at late stages.

Ma et al. [12] observed that nicotine could compromise bone formation and remodeling by affecting the expression of osteogenic and angiogenic growth factors. The results showed that nicotine inhibited the gene expression of BMP-2, TGF-b1 and PDGF-AA, and also the
expression of VEGF by osteoblasts. This activity might contribute to the failure of dental implant osseointegration in the presence of nicotine.

Yuhara et al. [47] examined the behavior of cultures of clonal rat calvarial osteogenic cells (ROB-C26), clonal mouse calvarial preosteoblastic cells (MC3T3-E1), and in osteoclast-like cells formed during coculture of mouse bone marrow cells and clonal stromal cells from mouse bone marrow, ST2 cells, in the presence of nicotine concentrations that occur in the saliva of smokeless tobacco users. Nicotine stimulated the rate of deposition of Ca\(^{2+}\), as well as the ALP activity by ROB-C26 cells, in a dose-dependent manner. However, both processes decreased in MC3T3-E1 cells that had been exposed to nicotine. In contrast, nicotine reduced, in a dose-dependent manner, the formation of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells (MNCs) and the formation of pits on slices of dentine, both of which are typical characteristics of osteoclasts. All this results suggest that nicotine might have critical effects on bone metabolism.

In Katono et al. [45] study, they have evaluated the combined effect of Lipopolysaccharide (LPS) from periodontopathic bacteria (which can initiate alveolar bone loss through the induction of host-derived cytokines) and nicotine, on the expression of matrix metalloproteinases (MMPs), plasminogen activators (PAs), and their inhibitors, including tissue inhibitors of metalloproteinases (TIMPs) and PA inhibitor-1 (PAI-1), in osteoblasts. Their results indicated that osteoblast proliferation was not affected by nicotine and LPS, but on the other hand, ALPase activity was significantly lower in cells cultured with nicotine or LPS than control, and the activity was even lower in cells cultured with both nicotine and LPS. This indicates that nicotine and LPS may suppress cell differentiation and mineralization of osteoblasts. Also, nicotine and LPS increased the production of MMPs and tPA and decrease the production of TIMPs, suggesting that these mechanism may contribute to the nicotine and LPS induced resorption that occurs during turnover of osteoid.

In this context, Shoji et al. [48] studied the effect of LPS and nicotine in Saos-2 human osteosarcoma cells (acting as osteoblasts). Their results suggest that nicotine and LPS might stimulate osteoclast formation through an increase in PGE2 and M-CSF production by osteoblasts. Furthermore, nicotine-and-LPS-induced PGE2 appeared to interact with the osteoblast Ep4 receptor, which may also enhance osteoclastogenesis by an increase in RANKL expression and a decrease in OPG production. Finally, they also reported that nicotine-and-LPS-induced PGE2 suppresses calcification by decreasing ALP activity.
Henemyre et al. [11] observed that nicotine elicited a significantly increase on osteoclast resorbing ability. However, no significant difference was found between the various nicotine doses. Nevertheless, the number of osteoclasts increased in a linear relationship to the increasing nicotine concentrations.

Pereira et al. [2] described the effect of nicotine in the behavior of HBM (human bone marrow) and Saos-2 osteoblastic cells, cultured on the surface of plasma-sprayed titanium implants. Their results showed that nicotine caused a dose-dependent effect on matrix mineralization of HBM and Saos-2 cells, reflected by an earlier onset of cell-mediated calcium phosphate deposition at levels up to 0.1–0.2 mg mL⁻¹.

In this context, the same authors [49] performed another study using only HBM cells. They observed that nicotine caused a dose-dependent effect on cell viability/growth and functional activity. A significant increase in cell growth rate was evident at levels up to 0.2mg/ml. Also, an induction in ALP activity and increased extent of matrix mineralization occurred in this concentration range, particularly in the presence of 0.05–0.2mg/ml nicotine. Their results strongly suggest a cell-mediated mineralization process occurring both in control and in nicotine-exposed seeded implants. In both studies, [2, 49], exposure of the seeded implants to nicotine caused a characteristic dose-dependent vacuolation of the cytoplasm that was particularly evident at concentrations higher than 0.2mg/ml.

It has been pointed out by authors that the differences among the effects of nicotine on osteoblast behaviour in vitro might reflect differences in culture conditions, differences among species, and the type of osteoblastic cell model used, including their stages of differentiation[12, 47]. Other limitation has to due with the great number of substances that constitute the particulate phase of tobacco smoke. In fact, more studies are needed to evaluate the influence of other tobacco components on bone metabolic processes.

In that context, Pereira et al. [50] evaluated the simultaneous effects of nicotine, acrolein, and acetaldehyde on osteogenic-induced HBM cultured on plasma-sprayed titanium implants. Their results showed that acrolein and acetaldehyde caused a dose-dependent inhibitory effects at levels similar to and greater than 0.03 and 0.1 mmol/L, respectively; IC50 (concentration that inhibits 50%) regarding cell viability/proliferation and ALP was 0.06 mmol/L for acrolein and 0.3 mmol/L for acetaldehyde. Matrix mineralization was prevented at levels higher than 0.03 mmol/L acrolein and 0.1 mmol/L acetaldehyde. The exposure to a combination of nicotine 1.2 mmol/L with acrolein (0.06 mmol/L), acetaldehyde (0.3 mmol/L), or both resulted in a cell behavior intermediate to that observed in nicotine-treated cultures.
(induced cell response) and aldehyde- treated cultures (deleterious cell response). On the other hand, exposure to nicotine 2.4 mmol/L with acrolein (0.06 mmol/L), acetaldehyde (0.3 mmol/L), or both caused cumulative cytotoxic responses. In summary, they suggest that interactions of tobacco compounds over osteoblasts might contribute to the overall effects of tobacco use on implant osseointegration and long-time survival.

**Conclusion**

There is a significant difference in the failure rates of dental implants between smokers and nonsmokers. Smokers have a higher incidence of failure and complications following dental implantation and implant-related surgical procedures. Although nicotine is considered a major compound in tobacco smoke, new information from recent studies suggest that it isn’t the unique responsible for the deleterious effect of smoking on the outcome of dental implants. In fact, it does affects bone cells metabolism, but the results of these studies give us mixed data, with both stimulatory and inhibitory effects.

In conclusion, more studies are needed, which must overcome the existing limitations (differences in culture conditions, differences among species, the type of osteoblastic cell model used, including their stages of differentiation, great number of substances that constitute the particulate phase of tobacco smoke) in order to attain true knowledge about the effect of nicotine in the osseointegration of bone implants.
References

Influence of nicotine in osteointegration of dental implants


