Effect of Smoking on Early Bone Healing Around Oxidized Surfaces: A Prospective, Controlled Study in Human Jaws

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Background: This prospective and controlled histologic study evaluates the impact of smoking on bone-to-implant contact, the bone density in the threaded area, and the bone density outside the threaded area around microimplants with anodized surface retrieved from human jaws.

Methods: A total of 24 subjects (mean age 51.32 ± 7.5 years) were divided in two groups: smokers (n = 13 subjects) and non-smokers (n = 11 subjects). Each subject received one microimplant with oxidized surface during conventional mandible or maxilla implant surgery. After 8 weeks, the microimplants and the surrounding tissue were removed and prepared for histomorphometric analysis.

Results: Three microimplants placed in smokers showed no osseointegration. The newly formed bone showed early stages of maturation, mainly in the non-smokers. Marginal bone loss, gap, and fibrous tissue were present around implants retrieved from smokers. Histometric evaluation indicated that the mean bone-to-implant contact ranged between 25.97% ± 9.02% and 40.01% ± 12.98% for smokers and non-smokers, respectively (P <0.001). Smokers presented 28.17% ± 10.32% of bone density in the threaded area, whereas non-smokers showed 46.34% ± 19.12%. The mean of bone density outside the threaded area ranged between 18.76% and 25.11% for smokers and non-smokers, respectively (P>0.05).

Conclusion: The present data obtained in human subjects confirm that smoking has a detrimental effect on early bone tissue response around oxidized implant surfaces. *J Periodontol 2010;81:575-583.*

KEY WORDS

Dental implants; histology; osseointegration; smoking; wound healing.

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Peri-implant bone healing is a complex phenomenon. This process involves a cascade of synthesis and activation of matrix proteins, growth factors, cytokines, and angiogenic stimulators that coordinate the restoration of mechanical stability of bone at the peri-implant interface.[1,2] However, smoking tobacco has been shown to be a risk factor for bone healing.[3,4]

The influence of smoking on peri-implant bone has been evaluated in several histologic animal models.[5-7] Most of these studies agree with the detrimental effect of smoking and its components on bone healing, bone-to-implant contact (BIC), and bone mineral density.

Smoking delays the normal bone healing process by a mechanism that inhibits proliferation of precursor cells essential to bone healing.[8] Cigarette smoking is composed of over 4,000 toxins that potentially undermine the peri-implant bone healing process. Toxins, such as nicotine, carbon monoxide, nitrosamines, benzenes, aldehydes, and hydrogen cyanide, have been shown to affect processes essential to bone healing.[9] Nicotine is a potent vasoconstrictor that reduces not only blood flow and nutrient delivery to the surgical implant site but also inhibits proliferation of fibroblasts, red blood cells, and macrophages.[10] Carbon monoxide

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decreases the oxygen-carrying capacity of red blood cells, whereas hydrogen cyanide leads to hypoxia.

Peri-implant bone healing might also be affected by the different dental implant surface topographies. In turn, each of these bone-healing processes is affected by physicochemical interaction between the molecules and cells in the surrounding peri-implant area. The implant surface topography properties and the specific properties of individual proteins determine the organization of the adsorbed protein layer.[2]

A plethora of researchers have evaluated several implant surface treatments, mainly anodic oxidation,[11-18] a surface topography modification technique that results in oxide layer growth to a thickness of 1 to 10 μm. This process provides topography with minimal to moderate roughness with numerous pores of varied sizes.[11,15] Studies that evaluated this implant surface topography have shown that BIC reacts to it more strongly than to machined implant surfaces.[13,17-19]

The purpose of the present prospective and controlled study is to evaluate bone reactions to oxidized surfaces after an unloaded healing period of 2 months in smokers and non-smokers.

MATERIAL AND METHODS

Selection of Subjects

Twenty-four partially edentulous subjects (14 women and 10 men), with a mean age of 51.32 ± 7.5 years, referred for oral rehabilitation with dental implants were included in this study (Table 1). The patients were divided into two groups: smokers (n = 11) and non-smokers (n = 13). Exclusion criteria included pregnancy, nursing, and any systemic condition that could affect bone healing. The Ethics Committee for Human Clinical Trials at Guarulhos University approved the study protocol (#201/03), which was explained to each subject, and all patients signed informed consent.

Calculation of the sample size was based on a series of studies published by Shibli et al.[16,19] A difference of 20% in BIC among implants of different surface topography was set because the present study design (smokers × non-smokers) is not available in the literature. With an α of 0.05 and 1-β of 0.80, a sample of at least 10 subjects per group was considered desirable.

Smoking

Patients were provided with a questionnaire to report their smoking history at baseline recruitment. They were asked to furnish information on smoking status (current, past, and never); frequency of smoking (number of cigarettes per day, week, or month); and number of years they smoked. The patients were categorized into smokers (>10 cigarettes a day for at least 5 years)[20] and non-smokers using the aforementioned information. The non-smoking group includes only those who never smoked to avoid bias in the present study design.

Table 1.

Clinical and Demographic Data

Variables	Smoker	Non-Smoker
Age (years)	50.6 ± 8.5	52 ± 5.2
Gender (male:female)	4:7	6:7
Partially edentulous	5	6
Totally edentulous	6	7
Microimplants placed in		
Posterior mandible	5	4
Posterior maxilla	8*	7

* Three implants placed in posterior maxilla failed and they were excluded from the analysis.

Oxidized surface

In this study, screw-shaped microimplants made of grade-4 titanium¶ were prepared with oxidized surface topography (Fig. 1). Each microimplant was 2.5 mm in diameter and 6 mm long.

The anodic oxidation method for the preparation of the oxidized implant surface topography has previously been described.[15,16] The screw-shaped implants were ultrasonically rinsed with acetone, pickled with a mixture of HF and HNO_3 (the HF/HNO_3 mole ratio was 1:3), and rinsed with distilled water. They were anodized with a regulated direct current power supply and a calcium glycerophosphate–calcium acetate electrolyte. Both calcium glycerophosphate and calcium acetate, typically used as food stabilizers and additives, are non-toxic and contain calcium with virtually no impurities. The specimens were rinsed several times with distilled water and dried after anodizing.

Implant Surface Topography

An optical laser profilometer# was used to measure and characterize the dental implant surface topography. Eight microimplants were measured three times each on the side, top, and bottom. The measured parameters, such as the arithmetic average of all profile point absolute values, the root-mean-square of all point values, and the average absolute height values of the five highest peaks and the depths of the five deepest valleys, were measured in all specimens.

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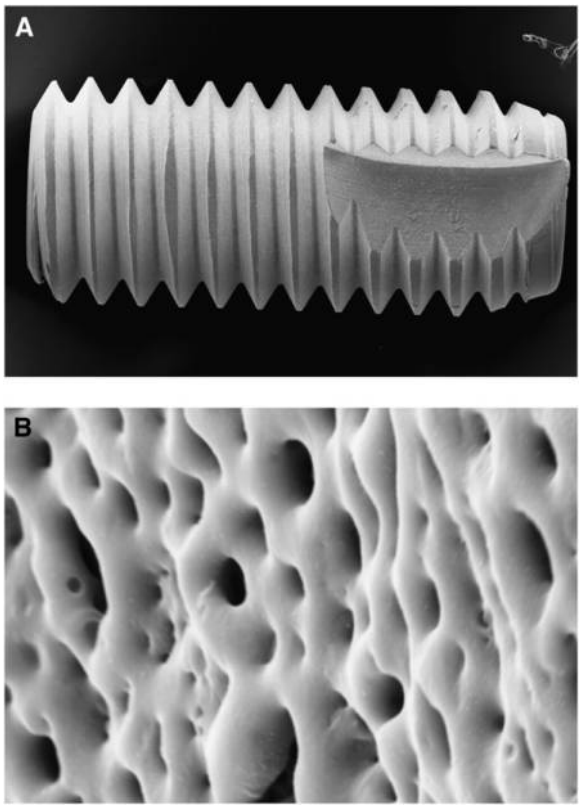

Figure 1.
A) *Scanning electron microphotograph of the microimplant.* ***B)*** *Details of the oxidized surface.*

In addition, an energy dispersive spectroscopy x-ray** coupled to the scanning electron microscope†† was used to evaluate the oxide layer of five microimplants. The spectral resolution of the detector was 138 eV at 5.7 kV (MnKa1). The microprobe used to acquire the spectra was set at 20 kV high tension, 750 mA probe current, and at several working distances.

Microimplant Surgery

Twenty-four screw-shaped microimplants were used in this study. All patients each received one microimplant. The microimplants were placed as previously reported.[17,19] After crestal incision, mucoperiosteal flaps were raised and conventional implants were placed in accordance with the surgical and prosthetic plan prepared for each patient. Next, the microimplants were placed in suitable areas, mostly in the molar region (i.e., posterior to the most distal conventional implant). The microimplant recipient sites were prepared with a 1.8-mm-diameter twist drill in soft bone and 2.2-diameter in dense bone. All drilling and microimplant placement procedures were completed under profuse irrigation with sterile saline. A backup surgical site was prepared if the microimplant showed low primary stability. The flaps were sutured to cover the microimplants. Clindamycin was administered three times a day for a week to avoid post-surgical infection. The sutures were removed after 10 days. To enable subjects to control postoperative dental biofilm, 0.12% chlorhexidine rinses were prescribed, twice a day for 14 days.

After a healing period of 2 months, the microimplants and the surrounding tissues were retrieved with a 4-mm-wide trephine bur, and the specimens were fixed by immediate immersion in neutral formalin at 4%.

Specimen Processing and Histometric Analyses

The biopsies were processed‡‡ to obtain thin ground sections as previously described.[21] The specimens were dehydrated in an ascending series of alcohol rinses and embedded in glycol methacrylate resin.§§ After polymerization, the specimens were sectioned lengthwise along the longer axis of the implant, using a high-precision diamond disk, to about 150 μm, and ground down to about 30 μm. One to two slides were obtained from each implant, and then averaged for each group. The slides were stained with basic fuchsin and toluidine blue. BIC% was defined as the amount of mineralized bone in direct contact with the implant surface. The measurements were made throughout the entire extent of the microimplant. The bone density in the threaded area (BA%) was defined as the fraction of mineralized bone tissue within the threaded area. All threads were measured. Bone density (BD%) in a 200-μm-wide zone lateral to the implant surface was measured bilaterally. These evaluations were performed using a light microscope connected to a high-resolution video camera and interfaced to a monitor and personal computer. This optical system was associated with a digitizing pad and a histometry software package with image-capture functionalities.‖‖ A single-blinded and calibrated examiner performed the histometric parameters. A total of 10 ground sections (five of non-smokers and five of smokers) were used for the calibration exercise. The sections were analyzed twice with a 1-week interval between measurements. Paired *t* test statistics showed no significant differences ($P>0.05$) in intraexaminer reproducibility. The standard errors of the mean differences of histometric analysis were 4%, 2.8%, and 6.54% for BIC, BA, and BD, respectively.

The mean and standard deviation of histometric variables were calculated for each implant, then for each group. Mann-Whitney U test was used to compare the differences between groups (smoker ×

** Energy dispersive espectroscopy (EDS)-ThermoNORAN Modelo QUEST, Noran Instruments, Middleton, WI.
†† SEM, JEOL Modelo 6360LV, JEOL, Tokyo, Japan.
‡‡ Precise 1 Automated System, Assing, Rome, Italy.
§§ Technovit 7200 VLC, Kulzer, Wehrheim, Germany.
‖‖ Image-Pro Plus 4.5, Media Cybernetics, Immagini and Computer, Milan, Italy.

non-smoker). The significance test was two-tailed and conducted at a 5% level of significance.

RESULTS

Surface Roughness Parameters

The oxidized surface showed no clear orientation. The anodic oxidation preparation provides an implant surface with a surface roughness with the mean ± SD of the absolute values of all profile points, the root mean square of the values of all points, and the average value of the absolute heights of the five highest peaks and the depths of the five deepest valleys of 0.87 ± 0.14, 1.12 ± 0.18, and 5.14 ± 0.69 μm, respectively.

Energy dispersive espectroscopy (EDS) analysis showed that all microimplants with oxidized surfaces consisted of 80.7% Ti oxide. The dominant element detected was oxygen (O), followed by P, Ca, Na, and Cl (Table 2).

Clinical Observations

Twenty-one microimplants were clinically stable at the time of retrieval. The microimplants were almost all placed in the posterior maxilla[22] (15 in total), being eight and seven in smokers and non-smokers, respectively. The remaining microimplants were placed in the posterior mandible. Only three microimplants placed in the posterior maxilla of smokers (two females) showed no osseointegration. These implants were not included in the authors' evaluation of the present study.

Histometric Results

The ground sections of both groups are presented in Figures 2 through 4. Tables 3 through 5 present the histometric variables. BIC% and BA% were significantly lower in smokers (P <0.05, Table 3). BIC% values for smokers ranged between 11.79% and 40.77%, whereas the means values for the non-smokers ranged between 14.89% and 63.34%. The mean BA% value for the smokers was 28.17%, ranging from 16.67% to 49.5%. The mean BA% value for the non-smokers was 46.34% ranging from 8.89% to 80.78%. The BD% in a 200-μm-wide zone lateral to the microimplant presented a mean of 18.76% and 25.11% for smokers and non-smokers, respectively (P >0.05).

Tables 4 and 5 show the mean histometric values for the implants placed in the maxilla and mandible, respectively. Smokers showed lower mean percentages for all histometric parameters. The mean BIC% value for the non-smokers (32.97% ± 8.98%) in the maxilla was higher (P = 0.03) compared to the values observed for the smokers (19.02% ± 4.38%). There was significant difference shown among the BIC% values for microimplants inserted in the mandible in both groups (P = 0.02).

Smoking influenced the BA% for microimplants retrieved from both the maxilla and mandible. The non-smokers presented higher mean BA% values than those obtained for the smokers. However, the mean BA% values for the maxilla and mandible did not differ significantly (P >0.05) for the groups (Tables 4 and 5).

In addition, when intragroup analysis was performed according to the microimplant position in the jaw (maxilla or mandible), the histometric data showed that both surfaces presented higher mean histometric values in the mandible (P >0.05) (data not shown).

Table 2.

Surface Composition of Microimplants With Oxidized Surfaces

Element	Weight (%)	Atomic Weight (Ar)	Molecular Formula	Oxide Proportion
O	40.24	64.96		0
Na	0.9	1.01	Na_2O	1.21
P	5.02	4.18	P_2O_5	11.49
Cl	2.38	1.74	Cl	2.38
Ca	3.46	2.23	CaO	4.83
Ti	48.01	25.88	TiO_2	80.07
Total	100	100		100

DISCUSSION

The present study evaluates the influence of cigarette smoking on early bone healing in human jaws. It was shown that smoking had negative effects on early osseointegration around oxidized implant surface topography assessed by histomorphometry, suggesting a clear tendency toward slower wound repair. These results confirmed previous animal studies that had shown that smoking interferes negatively either with BIC or guided bone regeneration.[5-7,23] To our knowledge, this is the first prospective controlled histologic study to investigate the impact of cigarette smoking on BIC in human jaws.

The lower percentage of BIC around the microimplants retrieved from smokers was the result of the interaction between cigarette smoking and host response. The peri-implant bone healing process is a coordinated process involving various biologic factors.[1] Indeed, many growth factors expressed during skeletal development and induced in response to injury are believed to regulate bone tissue repair.[24] Some of these molecules are also involved in angiogenesis.[25] The involvement of vascular growth factors

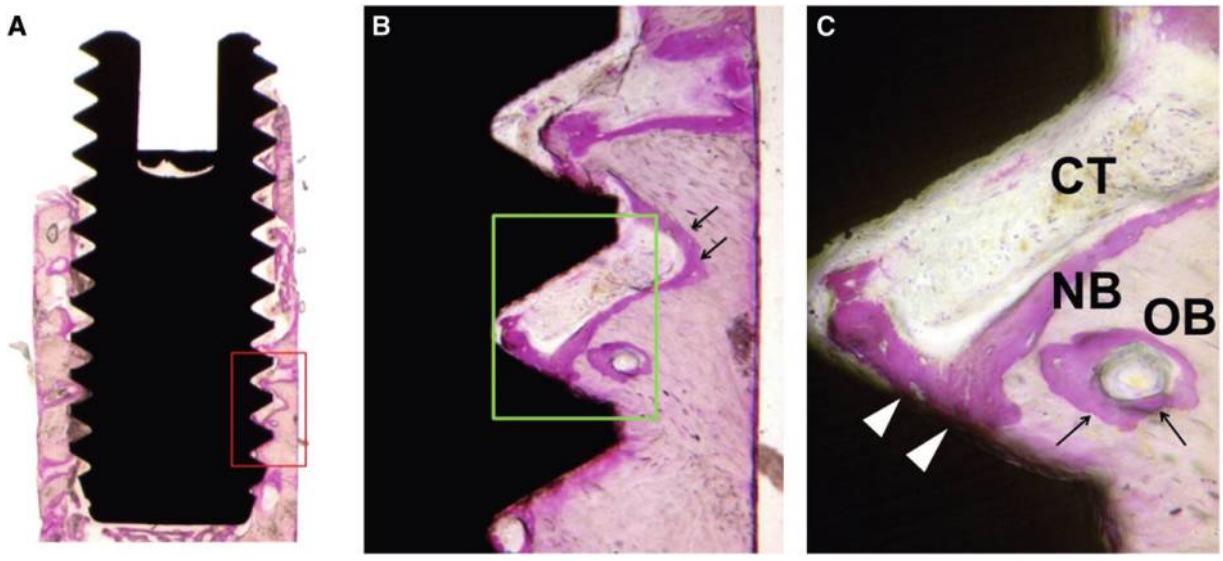

Figure 2.
A) *Histologic ground section of the microimplant retrieved after 8 weeks of healing from a posterior mandible of non-smoker depicting the newly formed bone showing early maturing and remodeling stages (basic fuchsin and toluidine blue, original magnification ×12).* ***B)*** *Higher power view of the lateral frame area in the section shown in A. The arrows show the reversal lines between newly formed bone and the older bone tissue. The newer bone tissue shows direct contact with the oxidized implant surface (basic fuchsin and toluidine blue, original magnification ×100).* ***C)*** *Larger magnification of the lateral frame area in the section shown in B. This view shows the presence of pristine (OB) and new bone (NB) and connective tissue (CT) inside of thread area. NB is in close contact with the oxidized implant surface (arrowheads). The arrows depict a primary harvesian channel (basic fuchsin and toluidine blue, original magnification ×200).*

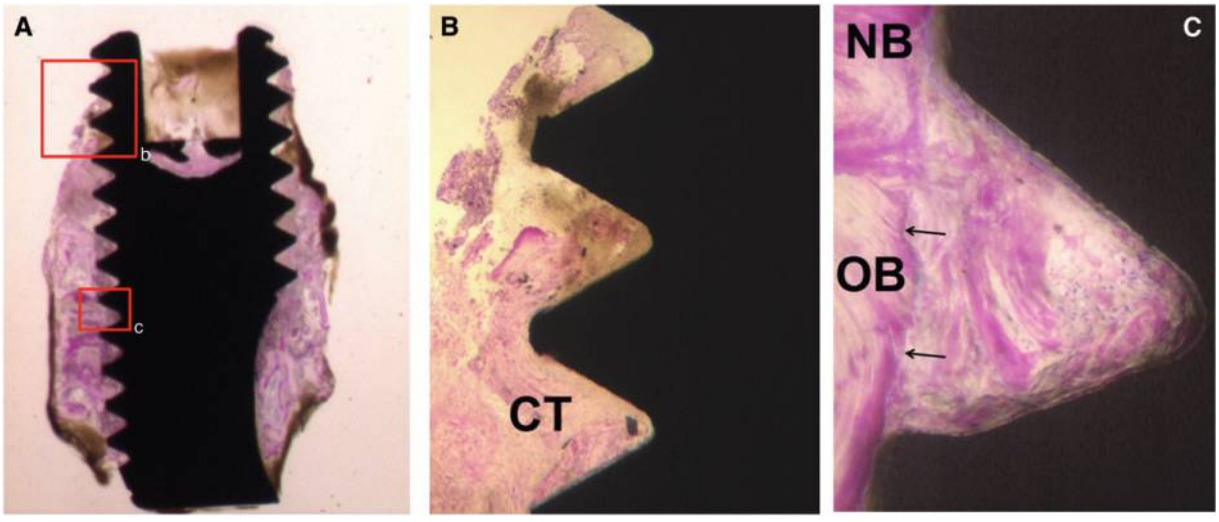

Figure 3.
A) *Histologic ground section of the oxidized microimplant surface depicting the newly formed bone shown at early maturing and remodeling stages retrieved from a posterior mandible of smoker (basic fuchsin and toluidine blue, original magnification ×12).* ***B)*** *Larger magnification of the lateral area in the section shown in A. Note the presence of marginal bone loss and connective tissue (CT) in contact with implant surface (basic fuchsin and toluidine blue, original magnification ×100).* ***C)*** *Higher magnification of the lateral area in the section shown in A. The arrows show the reversal line between pristine (OB) and a mixture of new bone (NB) and remaining bone from drilling process (basic fuchsin and toluidine blue, original magnification ×200).*

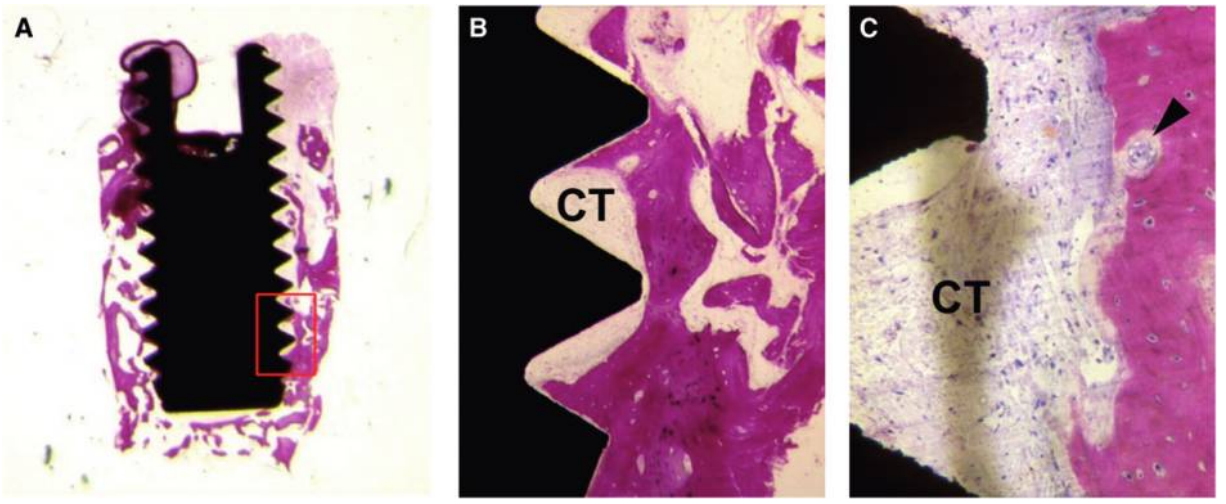

Figure 4.
A) *Histologic ground section of the microimplant retrieved after 8 weeks of healing from a posterior maxillae of smoker depicting the newly formed bone showing early maturing and remodeling stages (basic fuchsin and toluidine blue, original magnification ×12).* ***B)*** *Higher power view of the lateral frame area in the section shown in A. The newly formed bone tissue shows areas of direct contact with the oxidized implant surface, whereas in some areas there are also a lack of connecting bridges between new bone and implant surface (basic fuchsin and toluidine blue, original magnification ×100). CT = connective tissue.* ***C)*** *Gap and connective tissue (CT) are presented between newly formed bone and implant surface. CT was loose with scattered inflammatory cells. The arrowhead depicts an osteoclast (basic fuchsin and toluidine blue, original magnification ×200).*

in bone formation is also suggested by its interaction with humoral factors that regulate bone homeostasis of all profile point absolute values[26] and by its role, not only in bone angiogenesis but also in different aspects of bone development, including chondrocyte differentiation, and osteoblast and osteoclast recruitment.[25] Bone formation is closely linked to blood vessel invasion, and therefore angiogenesis plays a pivotal role in all regenerative processes.[1,24,27] On the other hand, smoking influences angiogenesis,[28] several aspects of leukocyte development, and function and host cytokine levels[29] that could, in part, explain the worst results observed for all histometric variables in the present study. Although these results focused on a single aspect (i.e., histometric comparison between smoker and non-smoker), and therefore the supposed mechanisms of smoking side effects on the peri-implant bone healing might not be completely discovered, an enhanced risk for peri-implant bone loss and implant loss could be expected. Therefore, a regular and strict recall of smokers undergoing implant treatment is needed for early detection of implant complications.[3]

Furthermore, it has been known that cigarette smoking not only reduces the rate of bone formation[30] but also increases the rate of bone destruction in postmenopausal women.[31] Imbalances between osteoclasts and osteoblasts can arise from several factors, such as hormonal changes, enhanced production of inflammatory cytokines, and growth factors that may result in decreased or increased bone mass. Earlier investigations[32] have shown that bone turnover is controlled by the interaction of the receptor activator of nuclear factor-kappa B ligand and osteoprotegerin (OPG). Receptor activator of nuclear factor-kappa B, receptor activator of the nuclear factor-kappa B ligand, and OPG are important in coordinating osteoclastogenesis and thereby alveolar bone resorption. In a similar manner, cigarette smoking and its components seem to suppress OPG levels and might contribute toward the decreased peri-implant bone formation, in agreement with the results of the present study and aforementioned animal studies.[5-7,23] However, the precise mechanisms by which smoking exerts its deleterious effects on bone healing remain unclear.

Long-term investigations have documented the high predictability of dental implants to restore partially and fully edentulous patients.[33,34] However, the survival data of dental implants placed in posterior maxilla were inferior to those placed in the anterior mandible where the bone density is frequently higher. In addition to influencing wound healing, cigarette smoking has also been implicated in decreasing bone density.[35,36] Therefore, smoking may also indirectly decrease implant success rates by giving rise to poor-quality bone, agreeing with the data of the present study, in which three implants placed in the posterior maxilla of smokers showed lack of osseointegration. Several authors[35-37] have also demonstrated that smokers had a significantly higher overall implant failure rate compared to non-smokers.

Marginal bone loss has been a common feature among smokers.[37] It was possible to observe early

Table 3.

Mean and Standard Deviation of Bone-to-Implant Contact Percentages (BIC%), Bone Density in the Threaded Area (BA%), and Bone Density (BD%) in a 200-μm-Wide Zone Lateral to the Microimplant for Smokers and Non-Smokers in Both Maxilla and Mandible*

Histometric Variables	Smokers† (n = 10 patients)		Non-Smokers (n = 11 patients)		*P* Value	95% Confidence Interval
	SD	Range	SD	Range		
BIC%	25.97 ± 9.02	11.79–40.77	40.01 ± 12.98	14.89–63.34	0.02	19.51–50.05
BA%	28.17 ± 10.32	16.67–49.5	46.34 ± 19.12	8.89–80.78	0.04	20.78–62.76
BD%	18.76 ± 8.97	12.45–37.1	25.11 ± 18.34	3.56–61.22	0.21	12.34–36.26

* Mann-Whitney U test ($P < 0.05$).
† Three failed implants were excluded from statistical analysis.

Table 4.

Mean and Standard Deviation of Bone-To-Implant Contact Percentages (BIC%), Bone Density in the Threaded Area (BA%), and Bone Density (BD%) in a 200-μm-Wide Zone Lateral to the Microimplant for Smokers and Non-Smokers in the Maxilla*

Histometric Variables	Smokers† (n = 5 patients)		Non-Smokers (n = 7 patients)		*P* Value	95% Confidence Interval
	SD	Range	SD	Range		
BIC%	19.02 ± 4.38	11.79–23.34	32.97 ± 8.98	14.89–39.88	0.03	13.57–41.28
BA%	20.30 ± 2.98	16.67–23.99	35.22 ± 16.39	8.89–58.88	0.01	16.60–50.38
BD%	14.01 ± 5.70	10–23.9	17.90 ± 8.38	3.56–29.89	0.20	6.90–25.65

* Mann-Whitney U test ($P < 0.05$).
† Three failed implants were excluded from statistical analysis.

Table 5.

Mean and Standard Deviation of Bone-To-Implant Contact Percentages (BIC%), Bone Density in the Threaded Area (BA%), and the Bone Density (BD%) in a 200-μm-Wide Zone Lateral to the Microimplant For Smokers and Non-Smokers in the Mandible*

Histometric Variables	Smokers (n = 5 patients)		Non-Smokers (n = 4 patients)		*P* Value	95% Confidence Interval
	SD	Range	SD	Range		
BIC%	32.93 ± 6.57	25.45–40.77	56.40 ± 8.38	47.09–63.34	0.02	24.76–77.22
BA%	36.03 ± 8.73	25.78–49.5	72.24 ± 11.40	59.29–80.78	0.03	25.19–100.5
BD%	23.51 ± 9.58	17.06–37.1	41.85 ± 16.9	29.90–61.22	0.14	–0.18–83.89

* Mann-Whitney U test ($P < 0.05$).

marginal bone loss with the presence of several bundles of connective tissue around the implant surface in some of the histologic slides in this study.

The oxidized implant surface topography evaluated in this study presented higher mean BIC% values in non-smokers ($P = 0.02$; Table 3), as previously demonstrated by earlier studies.[12,14,18] Although a recent study[38] has shown that the survival rate of implants with an oxidized surface was not affected by smoking, the data of the present study suggest a slower bone healing process, at least under unloaded conditions. However, it must be pointed out that the oxidized surface evaluated in the aforementioned study[38] underwent a different surface topography preparation from that applied to the implant surface in the present study.

CONCLUSIONS

Within the limits of this study, it can be assumed that cigarette smoking may influence bone healing around oxidized surfaces. However, these data should be considered with caution, and further prospective, controlled, and randomized studies evaluating the clinical and radiographic long-term success of implant-supported restorations must be conducted.

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