earlyosseointegration of dental implants:

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Abstract

Objective: This study evaluated the effect of cigarette smoking on the percentage of early bone-to-implant contact (BIC%), the bone density in the threaded area (BA%) as well as the bone density outside the threaded area (BD%) around micro-implants with sandblasted acid-etched surface retrieved from human jaws.

Material and methods: Twenty-two subjects (mean age 55.4 4.5 years) were divided in two groups: smokers (*n* = 11 subjects) and never-smokers (*n* = 11 subjects). Each subject received one micro-implant during conventional mandible or maxilla implant surgery. After 8 weeks, the micro-implants and the surrounding tissue were removed and prepared for histomorphometric analysis. Results: Two micro-implants placed in smokers showed no osseointegration. Early stages of maturation of the newly formed bone were present, mainly in the never-smokers. Marginal bone loss, gap, and fibrous tissue were present around some implants retrieved from smokers.

Histometric evaluation indicated that the mean BIC% ranged between 25.9 9.1 and 39.8 14.2 for smokers and non-smokers, respectively (*P* = 0.02). Smokers presented 28.6 10.1 of BA% whilenever-smokers showed 46.4 18.8 (*P* = 0.04). The mean of BD% ranged between 19.1 7.6 and 28.5 18.8 for smokers and never-smokers, respectively (*P* = 0.21).

Conclusion: Cigarette smoking has a detrimental effect on early bone tissue response aroundsandblasted acid-etched implant surface topographies.

Smoking tobacco has been shown to be a risk factor for peri-implant bone healing (Sen- nerby & Roos 1998; Strietzel et al. 2007; Shi- bli et al. 2010b). The detrimental effect of smoking and its components on bone-to-im-plant contact (BIC), bone mineral density, and bone healing has been evaluated in a sev-eral histologic animal models (Cesar-Neto et al. 2005a,b; Correa et al. 2009).

Cigarette smoking is composed of over 4700toxins that potentially undermine the peri-im-plant bone-healing process. Smoking delays the normal bone-healing process by a mecha-nism that inhibits proliferation of precursor cells essential to bone healing (Dahl & Toks- vig-Larsen 2004). Toxins such as nicotine, car-bon monoxide, nitrosamines, benzenes, aldehydes, and hydrogen cyanide have been shown to affect processes essential to bone healing (Yuhara et al. 1999). 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 (Yildiz 2004). Carbon monoxide decreases the oxygen carrying capacity of red blood cells while hydrogen cyanide leads to hypoxia.

On the other hand, peri-implant bone heal- ing might also be affected by the different dental implant surface topographies (Shibli et al. 2007a,b, 2010a). This complex 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 (Puleo & Nanci 1999; Davies 2007). Each of these bone-healing processes is affected by physico- chemical interaction between the molecules and cells around peri-implant area. The sur- face topography properties, as well as the specific properties of individual proteins, determine the organization of the adsorbed protein layer (Davies 2007

The dental implant quality depends on the chemical, physical, mechanical, and topo- graphic properties of the surface. These differ- ent properties interact and determine the activity of the cells close to the dental implant surface. The sandblasted acid-etched surface is obtained by treating the commer-cially pure titanium dental implant with a spray of air and abrasive material (aluminum oxide or titanium oxide) for a certain period of time and under controlled pressure.

Next, this modified surface is pickled with acid solutions under different temperatures and periods of time to remove any residue and to condition the blasted surface. In addition, the properties of this surface influence cell migration and proliferation, resulting in bet- ter BIC% (Shibli et al. 2007a, 2010b,c).

Few studies and case reports have been published in the last years to evaluate the peri-implant bone response in smokers (d'Avila et al. 2010; Shibli et al. 2010c); therefore, the quality of the bone-implant interface around sandblasted acid-etched sur- faces after a short period of healing on smok- ers is still to be determined.

The purpose of the present prospective study was to evaluate the impact of smoking on early human bone apposition around sand- blasted acid-etched surfaces after an unloaded healing period of 2 months.

Material and methods

Selection of subjects

Twenty-two partially or totally edentulous subjects (13 women; nine men), with a mean age of 56 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 patients) and never-smokers (n = 11 patients). Exclu- sion 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 the patients signed Informed Consent.

Smoking

At baseline recruitment, patients were pro- vided with a questionnaire to report their smoking history. They were asked to furnish information on smoking status (current, past, and never), frequency of smoking (number of cigarettes per day or week or a month), and number of years they smoked. Using the aforementioned information, the patients were categorized into smokers (>10 cigarettes a day for at least 5 years) and never-smokers (d'Avila et al. 2010; Shibli et al. 2010c). The never-smoking group includes only those who never smoked to avoid bias in this study design.

Micro-implants and implant surface topography

In this study, screw-shaped micro-implants with 2.5 mm in diameter and 6.0 mm long made of grade-4 titanium (Conexão Dental Implants, São Paulo, SP, Brazil) were prepared by sandblasted acid-etched surface technology as previously described (Grassi et al. 2006) (Fig. 1). Briefly, the acid-etched process (HCl/ HNO_3) was controlled to create a homoge- neous implant surface topography. The experi- mental implants were blasted with 25–100 lm TiO_2 particles. After sandblasting, the dental implants were ultrasonically cleaned with an alkaline solution, washed in distilled water, and pickled with a mixture of HNO_3 and HF.

The samples were first checked for chemical composition with XPS/ESCA (X-Ray Photo- electron Spectroscopy/Electron Spectroscopy for Chemical Analysis), and no significant pol- lution was detected. The topographies at the microscale were then visualized using routine scanning electron microscopy (SEM) control. At the nanoscale, the SEM confirmed that both surface types were nanosmooth (Dohan Ehrenfest et al. 2014).

An optical laser profilometer (Mahr GmbH, Brauweg 38 Gottingen, Germany) measured the micro-implant surface topography. The preparation process provides an implant sur- face with a surface roughness with the mean and standard deviation of the absolute values of all profile points (Ra), the root mean square of the values of all points (Rq) and the average value of the absolute heights of the five high- est peaks and the depths of the five deepest valleys (Rz) of 0.87 0.14, 1.12 0.18, and
5.14 0.69 lm, respectively.

Experimental design and surgical procedures

Twenty-two screw-shaped micro-implants were used in this study. Each subject received one micro-implant, inserted in the posterior region of the mandible or of the maxilla, always distal to the last conven-tional implant placed. The bone density was between type 4 and 5 according Cawood & Howell (1998).

The conventional dental implants were placed under aseptic conditions, after a cre-stal incision and the elevation of mucope- riosteal flaps. The surgical sites were prepared either with 1.8-mm-diameter twist drill, in the maxilla, or, a 2.0-mm twist drill in the mandible. Afterward, the micro-im-plants were inserted with a screwdriver. If the micro-implant presented low primary sta- bility and/or mobility, a second surgical site was prepared. Drilling procedures and micro- implant placements were made under profuse irrigation with sterile saline. The flaps were sutured with single

interrupted sutures, sub-merging the micro-implants. A total of 300 mg of clindamycyn was given three times a day for a week, to avoid post-surgical infection (Grassi et al. 2006). A total of 50 mg of potassic diclofenac was adminis- tered for pain control, three times a day for 5 days. Sutures were removed after 10 days. For postoperative dental biofilm control, sub- jects were prescribed 0.12% chlorhexidine rinses twice a day during 14 days.

After healing period of 2 months, the micro-implants and the surrounding tissues were retrieved with a 4.0-mm-wide trephine bur, and the specimens were fixed by imme- diate immersion in 4% neutral formalin.

Processing of specimens and histometric analysis

The biopsies were processed to obtain thin ground sections (Precise 1 Automated Sys-tem®, Assing, Rome, Italy) as previously described (Piattelli et al. 1997). The speci- mens were dehydrated in an ascending series of alcohol rinses and embedded in a glycol methacrylate resin (Technovit® 7200 VLC, Kulzer, Wehrheim, Germany). After polymer-ization, the specimens were sectioned longi- tudinally along the major axis of the implant with a high-precision diamond disk at about 150 lm and ground down to about 30 lm. One to two slides were obtained for each implant. The slides were stained with basic fuchsin and toluidine blue. BIC% was mea- sured around all implant surfaces. 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-lm-wide zone lateral to the implant surface was measured bilaterally. These evaluations were performed using a light microscope con-nected to a high-resolution video camera and interfaced to a monitor and personal com- puter. This optical system was associated with a digitizing pad and a histometry soft-ware package with image-capture functionali-ties (Image-Pro Plus® 4.5, Media Cybernetics Inc., Immagini & Computer Snc, Milan, Italy). The mean and standard deviation of histometric variables were calculated for each micro-implant, then for each group. Data were first examined for normality by the Kol-mogorov–Smirnov test (data not shown); as the data did not achieve normality, analysis was performed using nonparametric method. Mann–Whitney *U*-test was used to compare the differences between groups (smoker and never-smoker). The significance test was 2- tailed and conducted at a 0.05 level of signifi- cance. (Misch 1990) being seven and six in smokers and never-smokers, respectively. The remain- ing micro-implants were placed in the poste- rior mandible. Only two micro-implants placed in the posterior maxilla of smokers (one female and one male) showed lack of osseointegration. These implants were not included in the authors' evaluation of this study.

Histometric results

The ground sections of both groups are pre- sented in Figs 2 and 3. Tables 2–4 present the histometric variables. BIC% and BA% were significantly lower in smokers.

Tables 3 and 4 show the mean histometric values for the implants placed in the maxilla and mandible, respectively. Smokers showed lower mean percentages for all histometric parameters. There was significant difference shown between the BIC% values for micro- implants inserted in the maxilla as in the mandible in both groups ($P = 0.03$ and $P = 0.02$, respectively). The smoking influ- enced the bone density in the thread area (BA %) for micro-implants retrieved from both the maxilla and mandible ($P = 0.01$ and $P = 0.03$, respectively). The never-smokers presented higher mean BA% values than those obtained for the smokers.

In addition, when intra-group analysis was performed according to the micro-implant position in the jaw (maxilla or mandible), in all histometric data evaluated, showed values in mean 55% lower in the maxilla.

Discussion

This study showed that smoking had detri- mental effects on early peri-implant bone heal-ing around sandblasted acid-etched implant surface topography suggesting a clear tendency toward slower wound repair. These results confirmed previous animal and human studies that had shown that smoking interferes nega-tively either with BIC or guided bone regenera- tion (Cesar-Neto et al. 2005a,b; Correa et al. 2009; Shibli et al. 2010c).

The lower mean values of BIC around the micro-implants retrieved from smokers were probably resulting of the interaction between cigarette smoking components and host response. The peri-implant bone apposition process is a

coordinated process involving various biological factors (Puleo & Nanci 1999). Indeed, many growth factors, expressed during skeletal development and induced in response to injury, are believed to regulate bone tissue repair (Bayliss et al. 2006). Some of these molecules are also involved in angio- genesis (Dai & Rabie 2007). The involvement of vascular growth factors in bone tissue for- mation is also suggested by its interaction with humoral factors that regulate bone homeostasis (Peng et al. 2002) and by its role, not only in bone angiogenesis but also in dif- ferent aspects of bone development, includ- ing chondrocyte differentiation, and osteoblast and osteoclast recruitment (Bayliss et al. 2006). Bone formation is closely linked to blood vessel invasion, and therefore, angio- genesis plays a pivotal role in all regenerative processes (Eckardt et al. 2003; Breithaupt- Faloppa et al. 2006). On the other hand, cigarette smoking influences angiogenesis (Cooke & Bitterman 2004), several aspects of leukocyte development and function as well as host cytokine levels (Rawlinson et al. 2003) that could, in part, explain the worst results observed for all histometric variables in the present study.

Furthermore, it has been known that smoking not only reduces the rate of bone formation (Laroche et al. 1994; Gaston & Simpson 2007) but also increase the rate of bone destruction in post-menopausal women (de Valk-de Roo et al. 1997; Iqbal 2000). Imbalances between osteoclasts and osteo- blasts can arise from several factors such as hormonal changes, enhanced production of inflammatory cytokines, and growth factors that may result in decreased bone mass or increased bone mass. Earlier investigations (Leibbrandt & Penninger 2008) have shown that bone turnover is controlled by the inter- action of the receptor activator of the NF-kB ligand (RANKL) and osteoprotegerin (OPG). RANK, RANKL, 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 for- mation, in agreement with the results of the present investigation and aforementioned animal (Cesar-Neto et al. 2005a,b; Correa et al. 2009) and human (Shibli et al. 2010c) studies. 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 (Roos-Jansåker et al. 2006; Astrand et al. 2008). 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 (Bain & Moy 1993; De Bruyn & Collaert 1994). 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 two implants placed in the posterior maxilla of smokers showed lack of osseointegration. Several authors (Bain & Moy 1993; De Bruyn & Col- laert 1994; Roos-Jansåker et al. 2006; Balshe et al. 2008) have also demonstrated that smokers had a significantly higher overall implant failure rate when compared with never-smokers.

Marginal bone loss has been a common feature among smokers (DeLuca & Zarb 2006). In some of the histological slides in this study, it was possible to observe early marginal bone loss with the presence of sev- eral bundles of connective tissue around implant surface. The sandblasted acid-etched surface evaluated in this study presented higher mean %BIC values in non-smokers (P = 0.02, Table 2), as previously demon-strated by earlier studies (Grassi et al. 2006, 2007; Shibli et al. 2010a).

Although these results focused on single aspect, that is, histometric comparison between smoker and never-smoker, and therefore, the supposed mechanisms of smok- ing 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. There- fore, a regular and strict recall of smokers undergoing to implant treatment is pretty need for early detection of implant complica- tions (Aglietta et al. 2011).

Finally, it can be assumed that a cigarette smoking may influence early peri-implant bone healing, at least, around sandblasted acid-etched surfaces. However, this histomet- ric data with limited sample size should be considered with caution, and further prospec- tive, controlled and randomized studies eval- uating the clinical and radiographic long-

Results

Clinical observations

Twenty micro-implants were clinically stable at the time of retrieval. Thirteen micro-im- plants were placed in the posterior maxilla

term success of implant-supported restora- tions in smokers must be conducted.

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Variables	Smoker	Non-smoker
Age (years)	57.4 6.8	55.0 6.7
Gender (M:F)	4:7	5:6
Partially Edentulous	5	6
Totally Edentulous	6	5
Micro-implants placed in		
Posterior Mandible	5	4
Posterior Maxilla	6*	7

*Two Implants placed in posterior maxilla fail, and they were excluded from the analysis.

Table 1. Clinical and demographical data

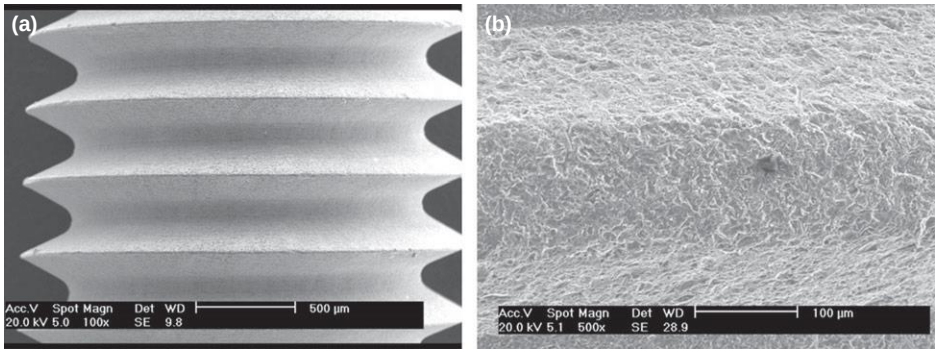

Fig. 1. (a) Scanning electron microphotograph of the micro-implant and threads and (b) details of the sandblasted acid-etched surface.

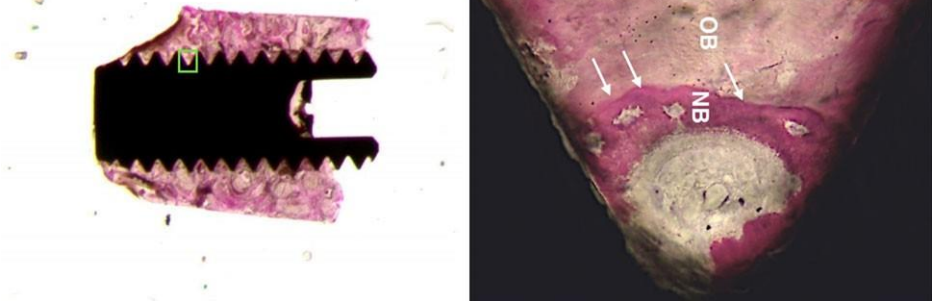

Fig. 2. (a) Histological ground section of the micro-implant retrieved after 2 months of healing from a posterior mandible of non-smoker depicting the newly formed bone showing early maturing and remodeling stages. (Basic fuchsin and toluidine blue staining, original 129 magnification); (b) higher power view of the section shown in (a). The arrows show the reversal lines between newly formed bone (NB) and the older bone (OB) tissue. The newer bone tissue shows direct contact with the sandblasted acid-etched surface. (Basic fuchsin and toluidine blue staining, original 1009 magnification).

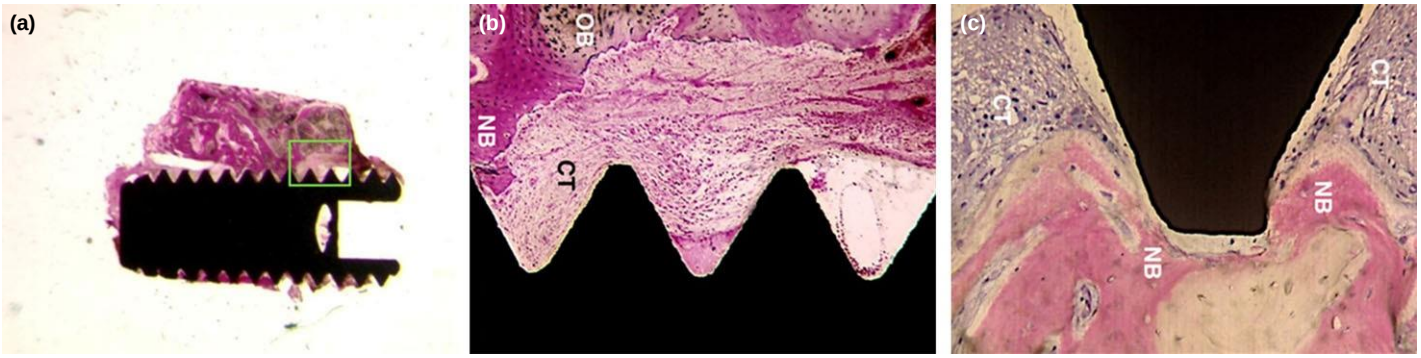

Fig. 3. (a) Histological ground section of the micro-implant retrieved after 8 weeks of healing from a posterior maxillae of smoker depicting the newly formed bone showing early maturing and remodeling stages. (Basic fuchsin and toluidine blue staining, original 129 magnification); (b) higher power view of the lateral frame area in the section shown in (a). The newly formed bone (NB) tissue shows areas of direct contact with the sandblasted acid-etched surface, although some areas there are also a lack of connecting brides between new bone and implant surface interposed by connective tissue (CT). (Basic fuchsin and toluidine blue staining, original 1009 magnification); (c) gap and connec- tive tissue (CT) are presented between newly formed bone (NB) and implant surface. CT was loose with scattered inflammatory cells. (Basic fuchsin and toluidine blue staining, original 2009 magnification).

Table 2. Mean standard deviation and median of bone-to-implant contact percentages (BIC%), bone density in the threaded area (BA%), and bone density (BD%) in a 200-lm-wide zone lateral to the micro-implant for smokers and never-smokers in both maxilla and mandible. Mann–Whitney *U*- test ($P < 0.05$)

	Smokers* (n = 9 patients)				Never-smokers (n = 11 patients)					
Histometric variables	Mean	SD	Median	Range	Mean	SD	Median	Range	P-value	CI 95% BIC%
25.94 9.14 23.38		11.88–38.76	39.82	14.24 37.83		10.85–63.34	0.02	19.51–50.05		
BA%	28.64	10.10	24.24	16.67–49.5	46.39	23.17	40.81	8.89–80.78	0.04	20.78–62.76
BD%	19.07	7.61	15.31	12.45–37.1	28.54	18.83	24.56	3.56–61.22	0.21	12.34–36.26

*Two failed implants were excluded from statistical analysis.

Table 3. Mean standard deviation and median of bone-to-implant contact percentages (BIC%), bone density in the threaded area (BA%), and bone density (BD%) in a 200 lm-wide zone lateral to the micro-implant for smokers and never-

smokers in the maxilla. Mann-Whitney *U* test ($P < 0.05$).

Smokers* (*n* = 6 patients)				Never-Smokers (*n* = 7 patients)					
Histometric variables	Mean	SD	Median	Range	Mean	SD	Median	Range	*P*-value	CI 95% BIC%
11.96	4.24	15.28	11.44–21.32	31.94	8.33	36.16	10.89–39.32	0.03	12.48–42.50	
BA%	13.25	2.38	18.78	18.33–22.70	32.93	13.66	38.99	8.44–55.11	0.01	16.37–51.29
BD%	10.29	5.10	11.30	10.16–22.45	17.68	9.23	17.87	4.76–27.22	0.20	7.86–23.56

*2 failed implants were excluded from statistical analysis.

Table 4. Mean standard deviation and median of bone-to-implant contact percentages (BIC%), bone density in the threaded area (BA%), and the bone density (BD%) in a 200 lm-wide zone lateral to the micro-implant for smokers and never-smokers on the mandible. Mann-Whitney *U* test($P < 0.05$).

	Smokers (*n* = 5 patients)				Never-smokers (*n* = 4 patients)					
Histometric variables	Mean	SD	Median	Range	Mean	SD	Median	Range	*P*-value	CI 95%
BIC%	32.33	8.32	32,27	25.12–38.92	53.55	8.42	52,93	45.00–61.99	0,01	22.34–74.44
BA%	35.66	8.30	34.77	25.19–38.77	69.63	11.45	69.83	45.00–81.02	0.02	24.22–100.1
BD%	22.32	10.56	21.22	17.27–31.31	40.36	16.23	47.83	29.70–63.90	0.25	–0.14–76.09