Histometric evaluation of bone around titanium implants with different surface treatments in rats exposed to cigarette smoke inhalation

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Abstract

Objectives: There is a lack of histological information about the influence of cigarette smoke on bone around surface-treated implants. The aim of the present study was to test the influence of titanium surface treatment on osseointegration in animals that were exposed to intermittent cigarette smoke inhalation.

Material and methods: Twenty-two male Wistar rats were used. One tibia, chosen at random, received a machined titanium implant (MI) while the other received an aluminum oxide-blasted surface implant (ABI). The animals were randomly assigned to one of the following groups: Group 1 – control (n = 11) and Group 2 – intermittent cigarette smoke inhalation (n = 11). Sixty days after surgery, the animals were sacrificed. The degree of bone-to-implant contact (BIC), bone filling (BF) within the limits of the threads of the implants and bone density (proportion of mineralized bone in a 500-μm-wide zone lateral to the implant – BD) were measured in the cortical (zone A) and cancellous bone (zone B) areas.

Results: Data analysis showed significant differences when comparing the groups and implant surfaces in both zones for BIC (two-way ANOVA – P < 0.05). The two groups presented higher BIC mean values for ABI, when compared with MI (P < 0.05). In group 2, cigarette smoke inhalation negatively affected BF in both zones (P < 0.05). Group 2 presented a significantly decreased BD in both zones (P < 0.05). No statistically significant differences were observed between surfaces in any of the groups for BD.

Conclusion: Within the limits of the present study, it can be concluded that the aluminum oxide blast surface treatment may increase the degree of BIC but cannot overcome the detrimental effect of tobacco smoke on bone around titanium implants.

The long-term success of implant therapy has been reported by several authors (Brånemark 1983; Lindquist & Carlsson 1985; Albrektsson et al. 1986; Zarb & Schmitt 1990). However, some patients lose a disproportionately high number of implants. This clustering of failure has been a limitation of implantology and several studies have investigated the factors that may favor such phenomena (Esposito et al. 1998; Lemmerman & Lemmerman 2005). Some local, behavioral and systemic conditions have been correlated with higher rates of failure, and smoking has been one of the most widely discussed factors [Lemmerman & Lemmerman 2005]. Clinical studies have reported that smokers present not only higher rates of implant failure than non-smokers [Bain & Moy 1993; De Bruyn & Collaert 1994; Lambert et al. 2000] but also a greater detrimental effect around successfully integrated implants [Haas et al. 1996; Lindquist et al. 1997; Roos-Jansaker et al. 2006], such as a higher
incidence of peri-implant mucositis and peri-implantitis and an increased marginal bone loss [Strietzel et al. 2007; Heitz-Mayfield 2008]. A recent meta-analysis, evaluating 19 appropriately selected studies, reported that when smokers were compared with non-smokers the odds ratio of implant failure was significantly elevated [OR = 2.17, 95% confidence intervals, 1.67–2.83] [Hinode et al. 2006]. In addition, a series of histological studies have documented a negative influence of smoking on bone healing around titanium implants inserted in rats [Nociti et al. 2002a, 2002b, César-Neto et al. 2003].

Quitting smoking is the most effective way to reduce tobacco’s harmful effects. In the only clinical study examining the impact of smoking interruption on implant outcomes, Bain [1996] evaluated a cessation protocol in which potential implant patients who smoked were encouraged to stop for 1 week before and 8 weeks after implant placement. The author found no difference in the failure rate between non-smoking controls and the smokers who quit the habit, whereas a significant difference was found between the continuing smokers and smokers who followed the cessation protocol. Histological studies of our group have also shown a beneficial effect of both temporary and complete smoking cessation on bone around titanium implants inserted in the rat tibiae and on tooth-supporting bone [César-Neto et al. 2005a, 2005b, 2006]. However, smoke quitting rate is very low because of nicotine’s high addiction potential. The smoking cessation rate is around 35–40% even with adjunct medication [Killen et al. 2006] and specialized follow-up [Akkaya et al. 2006].

With the objective of reducing the negative impact of smoking on implant outcomes, without the difficulties related to smoking cessation, some authors have proposed the use of surface-treated titanium implants [which have properties that may stimulate bone formation]. Kumar et al. [2002] evaluated the effect of smoking on achieving initial osseointegration in patients rehabilitated with surface-treated implants. Ninety-seven percent of the implants placed in smokers, and 98.4% of implants placed in non-smokers osseointegrated successfully. A similar figure was observed by Bain et al. [2002], who examined the influence of smoking on integration success and the longevity of machined [n = 2614] and acid-etched titanium implants [n = 2288]. Although there was no significant difference between smokers and non-smokers in either type of surface, within the smoking sub-group, the cumulative success rates were 93.5% for the machined implants and 98.7% for the acid-etched implants.

Based on the clinical significance of this topic and the lack of histological information about the influence of cigarette smoke on bone around surface-treated implants, the present study tested the influence of titanium surface treatment on osseointegration in animals that were exposed to intermittent cigarette smoke inhalation.

Material and methods

Animals

Twenty-two male Wistar rats [300–400 g] were included in the study. The animals were kept in plastic cages with access to food and water ad libitum. Before the surgical procedures, all animals were allowed to acclimatize to the laboratory environment for a period of 5 days. The protocol was approved by the University of Campinas Institutional Animal Care and Use Committee.

Implant surgery

General anesthesia was carried out by intramuscular administration of ketamine [0.5 ml/kg]. The skin was cleansed with iodine surgical soap. An incision approximately 1 cm in length was made in both the tibiae and the bone surface, which surgically exposed by blunt dissection. Under profuse saline irrigation, bicortical implant beds were drilled at a rotary speed not exceeding 1,500 rpm. One tibia, chosen at random, received a screw-shaped machined titanium implant 4 mm in length and 2.2 mm in diameter (ML) while the other received a similar implant with the surface blasted with aluminium oxide (ABI). Finally, soft tissues were replaced and sutured. Postoperatively, the animals received an antibiotic [Penbatióico, Wyeth-Whitehall Ltda, São Paulo, SP, Brazil] administered through a single intramuscular injection.

Experimental design

After the implant surgery, the animals were randomly assigned to one of the following treatment groups: Group 1 – control (n = 11) and Group 2 – intermittent cigarette smoke inhalation (n = 11). Group 2 animals were intermittently housed in an animal cigarette smoke exposure chamber as described previously [Nociti et al. 2002a, 2002b, César-Neto et al. 2003, 2005a, 2005b, 2006]. Briefly, the device consisted of a 45 × 25 × 20 cm³ clear acrylic chamber, an air pump and two inflow/outflow tubes. Five animals [group 2] were housed in the chamber at the same time, and the cigarette smoke of 10 cigarettes, containing 1.3 mg of nicotine each, was pumped into the chamber. Thus, the animals breathed the cigarette smoke that contaminated the air for 8 min, three times daily, until they were sacrificed [60 days]. The animals of group 1 were not exposed to the cigarette smoke at anytime.

Histometric procedure

After 60 days, the animals were sacrificed, the tibiae were removed and fixed in 4% neutral formalin for 48 h. Undecalcified sections were prepared as described previously [Nociti et al. 2002a], i.e. the blocks were dehydrated using an ascending series of ethanol [60–100%] and embedded in glycolmethacrylate [Technovit 7200”; Heraeus Kulzer GmbH, Wehrheim, Germany]. Subsequently, the sections [20–30 μm] were obtained in the long axis of the implants [longitudinal], perpendicular to their threads, and stained with toluidine blue 1%. One section representative of the implant mid-portion was used for histometric analyses. A single blind examiner performed the histometric analysis using an image analysis system [Image-Pro”; Media Cybernetics, Silver Spring, MD, USA]. The percentage of bone-to-implant contact (BIC) was obtained by the ratio of the extent of bone in direct contact with the implant threads and the total extent of the implant thread surfaces, multiplied by one hundred. Both the percentages of bone density [BD] [proportion of mineralized bone in a 500-μm-wide zone lateral to the implant] and bone filling [BF] within the threads of the implants were obtained by the point-counting technique [Nociti et al. 2002a, 2002b]. Data were arranged separately in cortical [zone A] and cancellous...
bone (zone B) areas, as described previously [Nociti et al. 2002a, 2002b; César-Neto et al. 2003].

Statistical analysis
A two-way analysis of variance (α = 0.05) was used to test the influence of cigarette smoke inhalation, implant surface design and its interaction on each histometric parameter (i.e. BIC, BD, and BF), separately, for zone A (cortical bone) and zone B (cancellous bone). Pairwise multiple comparisons were carried out by the Tukey test (α = 0.05) when the two-way ANOVA test detected significant differences.

Results
Histometric analysis
Statistical analysis revealed a significant difference regarding BIC on comparing groups and surface treatment in both zones (P < 0.05) [Fig. 1]. Group 1 and group 2 presented higher BIC mean values for ABI, when compared with MI (P < 0.05). With respect to BF of the threads, an influence of cigarette smoke inhalation was noted on both MI and ABI (P < 0.05) [Fig. 2]. Group 2 showed a significant decrease in BD in both zones (P < 0.05). No statistically significant differences were observed between surfaces in any of the groups for BD [Fig. 3]. Figures 4 and 5 show the histological aspects of the experimental groups.

Discussion
Recent clinical studies have suggested that the use of surface-treated implants in smokers could decrease the harmful effect of cigarette consumption on implant success rates [Bain et al. 2002; Kumar et al. 2002]. Preliminary data have shown promising results, with surface-treated implants presenting higher success rates than the machined implants in smokers [Bain et al. 2002]. In light of these results, the present study investigated whether the histological aspect of bone around ABI compared with MI might support the clinical findings of surface-treated implants in smokers. In this regard, a protocol previously used to histologically document the impact of nicotine and cigarette smoke on bone around titanium implants was adopted [Nociti et al. 2002a, 2002b; César-Neto et al. 2003, 2005a, 2005b, 2006]. It is necessary to recognize that the impact of smoking on bone around titanium implants is probably stronger in the mouth than in the tibiae, once the physical presence of smoke particles, the heat and probably a higher concentration of toxic compounds of cigarette smoke are observed in the oral environment of smokers [Bain & Moy 1993; Levin & Schwartz-Arad 2005; Wang et al. 2006; Feuerstein et al. 2008]. These factors may increase the risk of implant failure in intra-oral sites, when compared with extra-oral sites.

The results of the present study suggest that the titanium surface treatment may enhance BIC even with the exposure to cigarette smoke. This finding is in agreement with a previous study in our laboratory using rabbits that received nicotine...
injections [Stefani et al. 2002]. Although in that study no effect of nicotine was observed on the histometric parameters evaluated [i.e. BIC and BF] for both implant types, it was observed that modified implant surfaces enhanced osseointegration in the nicotine group. These results may help to explain the clinical data provided by Kumar et al. [2002] and Bain et al. [2002], who did not observe a negative influence of smoking on rough-surface implants. However, these initial clinical results may be interpreted with caution since Kumar et al. [2002] evaluated the effect of smoking on initial osseointegration and the follow-up period of Bain et al. [2002] was 3 years. The results of the present study may reinforce this caution because both surfaces were negatively influenced by the exposure to intermittent cigarette smoke inhalation.

The ideal strategy to avoid the tobacco effects of tobacco is definitively interrupting cigarette consumption. Recent findings showed that both temporary and complete cigarette smoke inhalation cessation reversed the negative effect of smoke exposure on bone healing around titanium implants [César-Neto et al. 2005a, 2005b]. Clinical data of Bain [1996] found no difference in the failure rate between non-smoking controls and smokers who quit, whereas a significant difference was noted between continuing smokers and smokers who followed the cessation protocol. Nevertheless, the adherence to quitting smoking programs is low, and individuals often revert to their smoking status. Data from Bain’s study [1996] (where all patients were advised to stop smoking) showed that 51 patients started on the cessation protocol and, 1 year later only 23 patients remained without smoking. In a recent study testing bupropion [the most effective non-nicotine-based cessation drug] as adjunct therapy for smoking cessation, it was observed that the abstinence percentages after 52 weeks were 33% and 34% for bupropion and placebo groups, respectively [Killen et al. 2006]. The smoking cessation rate is also low in patients treated in specialized clinics (where patients have professional support and medication such as nicotine patches and bupropion when necessary). In an evaluation of 349 patients who completed a 1-year follow-up of a smoking cessation program, it was found that only 151 (43.26%) were successful in quitting [Akay et al. 2006]. Because smoking cessation is difficult to achieve for the majority of the patients, the use of surfacetreated implants in smokers could help to decrease the negative impact of smoking consumption on implant success rates. Despite the great number of commercially available surface configurations, in this study rough-surface implants were compared with machined-surface implants. Thus, more investigations are needed to verify which surface would provide the best results under these conditions.

It is well established that the titanium surface structure plays an important role in implant fixation and biological interaction with surrounding tissues [Kasemo 1983]. The surface roughness may have direct effects on osteoblast migration, attachment, proliferation, and differentiation [Bachle & Kohal 2004]. It may also influence bone

Fig. 4. Photomicrograph illustrating the histological aspect observed around the implants placed in the animals of group 1. (A) Machined titanium implant. (B) Aluminum oxide-blasted surface implant. Toluidine blue/original magnification = × 6.25. Scale bar = 0.5 mm.

Fig. 5. Photomicrograph illustrating the histological aspect observed around the implants placed in the animals of group 2. (A) Machined titanium implant. (B) Aluminum oxide-blasted surface implant. Toluidine blue/original magnification = × 6.25. Scale bar = 0.5 mm.
organization [Wennerberg et al. 1996; Kim et al. 2003] and bone formation velocity [Cochran et al. 2002]. In the present study, it was observed that surface characteristics produced by aluminum oxide blast supported a greater BIC on the threads when compared with machined surfaces. This result is in accordance with histological [Wennerberg et al. 1996; Kim et al. 2003] and in vitro [Sader et al. 2005] studies that previously showed a positive impact of aluminum-blasted titanium surfaces in the interaction with bone tissue and osteoblast cultures. These findings additionally suggest that the biological properties of ABIs are also important to favor osseointegration in the presence of tobacco compounds. In vitro studies should be performed in order to better investigate the behavior of osteoblasts in the presence of tobacco compounds. This kind of study would be of substantial importance to direct future research and to complement clinical and histological findings.

There are a huge number of commercially available surface configurations that may vary considerably depending on the surface treatment of the implant [Ellingsen 1998]. Variation of the surface microstructure has been reported to influence the stress distribution, retention of the implants in bone and cell responses to the implant surface [Ellingsen 1998]. However, the ideal titanium surface treatment is still controversial and there is no consensus on which surface is indicated for each clinical situation. The present findings may raise the question of which surfaces would have the best properties to be used in the presence of tobacco compounds. There are other aspects to be considered like the degree of tobacco consumption (i.e. low, moderate, and heavy smokers), quality of surrounding bone and influence of the surface on biofilm accumulation, when exposed. Prospective clinical trials to evaluate the outcomes of surface-treated implants in smokers on a long-term basis are urgently required.

Although the implant surface treatment increased the degree of BIC in the presence of cigarette smoke compounds, intermittent cigarette smoke inhalation had a significantly detrimental effect on BF of the threads and the proportion of mineralized tissue for machined and rough implants. Therefore, this particular surface treatment may not be enough to overcome the negative effect of smoking on bone around titanium implants.

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References


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