Bone Density Around Titanium Implants May Be Influenced by Intermittent Cigarette Smoke Inhalation: A Histometric Study in Rats

Francisco Humberto Nociti, Jr, DDS, MS, PhD¹/João Batista César Neto, DDS²/ Marcelo Diniz Carvalho, DDS²/Enilson Antonio Sallum, DDS, MS, PhD¹

Purpose: This study investigated the influence of cigarette smoke on bone healing around titanium implants placed in rats. Materials and Methods: After administration of anesthesia, the tibia surface was exposed and screw-shaped titanium implants (4.0 mm in length and 2.2 mm in diameter) were placed bilaterally (1 each side). The animals (n = 32) were randomly assigned to either group 1 (control, n = 18) or group 2 (intermittent cigarette smoke inhalation, n = 14). After 60 days, the animals were sacrificed and undecalcified sections obtained. Bone density (the proportion of mineralized bone in a 500-µm-wide zone lateral to the implant) was measured in the cortical (zone A) and cancellous bone (zone B) areas. Results: In zone A, a slight difference in bone density was noted between the groups (96.18% ± 1.08% and 95.38 ± 1.17% in groups 1 and 2, respectively; P > .05) but was not statistically significant. In contrast, bone density was significantly decreased in zone B in the animals that were exposed to cigarette smoke (17.57 ± 6.45% and 11.30 ± 6.81% for groups 1 and 2, respectively; P < .05). Discussion: Whether different results would be observed if animals were exposed to cigarette smoke for a longer period of time and/or before implant placement remains to be investigated. Conclusion: Although intermittent cigarette smoke exposure may not seriously affect cortical bone density, it may jeopardize bone quality around titanium implants in the cancellous bone area. (Int J Oral Maxillofac Implants 2002;17:347–352)

Key words: dental implants, nicotine, osseointegration, smoking
maxillary implants. Lindquist and colleagues\textsuperscript{16} investigated the influence of smoking and other possibly relevant factors on bone loss around mandibular implants. They demonstrated that smoking was the most important factor affecting the rate of peri-implant bone loss. Esposito and coworkers\textsuperscript{5} reviewed the literature regarding factors associated with the loss of oral implants and concluded that smoking was one of the factors associated with biologic failures of implants. Recently, Lambert and associates\textsuperscript{17} reported long-term clinical outcomes of dental implants placed in smokers and nonsmokers in a longitudinal clinical study. The authors concluded that smoking promoted an increased implant failure rate.

In addition to the clinical reports related to the influence of smoking on bone healing around titanium implants, Stefani and colleagues\textsuperscript{18} investigated the effect of nicotine administration on the osseointegration process around dental implants. A slight negative effect of nicotine on the bone-to-implant contact around implants with machined surfaces was observed, although this difference was not statistically significant. At that time, it was stated that nicotine, by itself, did not interfere with bone healing around titanium implants.

To date, no information is available, at an experimental level, regarding the effect of cigarette smoke as a whole on the osseointegration process. Therefore, the present study was designed to evaluate, by histologic analysis, the influence of cigarette smoke on bone healing around titanium implants placed in the tibiae of rats.

MATERIALS AND METHODS

**Animals**

Thirty-two male Wistar rats (300 to 400 g) were used in the study. The animals were kept in plastic cages with access to food and water ad libitum. Prior to the surgical procedures, all animals were allowed to acclimate 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 obtained by the 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, and the bone surface of the tibiae was surgically exposed by blunt dissection. Under profuse saline irrigation, bicortical implant beds were drilled at a rotary speed not exceeding 1,500 rpm, and 1 screw-shaped commercially pure titanium implant (designed for this study), 4.0 mm in length and 2.2 mm in diameter, was placed bilaterally until the screw thread had been completely introduced into the bone cortex. Finally, soft tissues were replaced and sutured. Postoperatively, the animals received an antibiotic (Pentabiotic, 1 mL/kg, Wyeth-Whitehall, São Paulo, Brazil) given as a single intramuscular injection.

**Experimental Design**

Immediately after the implant surgery, the animals were randomly assigned to 1 of 2 treatment groups: group 1: control (n = 18); or group 2: intermittent cigarette smoke inhalation (n = 14). All animals of group 2 were intermittently housed in an animal cigarette smoke exposure chamber (Fig 1) for 8 minutes 3 times daily until they were sacrificed (60 days).

The animal cigarette device was designed specifically for this investigation. It was composed of a 45\times25\times20-cm\textsuperscript{3} clear acrylic resin chamber, an air pump, and 2 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 were forced to breathe the cigarette smoke that contaminated the air for 8 minutes. The animals of group 1 were not exposed to the cigarette smoke at any time.

**Histometric Procedure**

After 60 days, the animals were sacrificed, and the tibiae were removed and fixed in 4% neutral formalin for 48 hours. Undecalcified sections were prepared as previously described,\textsuperscript{19} ie, the blocks were dehydrated by using an ascending series of ethanols (60% to 100%) and embedded in glycolmethacrylate resin (Technovit 7200, Heraeus Kulzer, Wehrheim, Germany). Subsequently, sections (20 to 30 µm each) were obtained and stained with 1% toluidine blue. Bone density (ie, the proportion of mineralized bone in a 500-µm-wide zone lateral to the implant) was determined bilaterally (Image-Pro, Media Cybernetics, Silver Spring, MD) in the cortical (zone A) and cancellous bone (zone B) areas (Fig 2).

**Statistical Analysis**

The data from zones A and B (cortical and cancellous bone, respectively) were averaged separately. The hypothesis that there was no influence of intermittent cigarette smoke inhalation on the bone density around the implants was tested by intergroup analysis (Mann-Whitney test; alpha = .05), ie, zone A (group 1) versus zone A (group 2) and zone B (group 1) versus zone B (group 2).
RESULTS

Clinical Observations
At the beginning of this investigation, a total of 36 animals were used. However, 4 animals from group 2 died as a consequence of exposure to the cigarette smoke. Most of the deaths occurred during the first 2 days of exposure. After this period, the animals that survived and were housed in the chamber for exposure to cigarette smoke demonstrated some breathing problems. In addition, a non-significant weight loss in the group 2 animals was detected.

Bone Density Measurements
Statistical analysis did not reveal significant differences between groups 1 and 2 with respect to bone density at the cortical bone area (zone A) (96.18 ± 1.08% and 95.38 ± 1.17% for groups 1 and 2, respectively; \( P > .05 \)). In contrast, a significant difference was observed between groups 1 and 2 regarding bone density at the cancellous bone area (zone B) (17.57 ± 6.45% and 11.30 ± 6.81% for groups 1 and 2, respectively; \( P < .05 \)). Figures 3 to 5 illustrate the histologic results for the experimental groups.

DISCUSSION

The present investigation is part of a series of studies that has tried to document, at the histologic level, the influence of consumption of cigarettes and/or their compounds on periodontitis progression and bone healing around titanium implants.

Based on all epidemiologic and clinical studies that classified smoking as a risk factor for periodontitis progression, the authors first reported in vivo the influence of nicotine administration on the progression rate of ligature-induced periodontitis in rats.20 Later, the influence of nicotine administration on bone healing around titanium implants placed in the tibiae of rabbits was evaluated histometrically.18 A tendency for a lower percentage of bone-to-implant contact in the group that received nicotine daily was observed; however, this difference was not statistically significant.

In addition, it has been demonstrated that the implant surface may exert a positive role in the percentage of bone-to-implant contact in subjects that receive nicotine. Lambert and associates17 reported clinically higher success rates for HA-coated implants in smokers compared to machined-surface implants. Nicotine is one of the 2,000 potentially toxic substances in tobacco smoke and has been demonstrated, in vivo and in vitro, to influence many biologic events.20-24 Despite this fact, within the limits of a previous study,18 it was hypothesized that nicotine would not influence bone healing around titanium implants by itself and that the adverse effects of cigarette consumption on the success rates of titanium implants would be related only to the cigarette smoke as a whole. Therefore,
the present study was proposed to investigate whether cigarette smoke inhalation would interfere with the bone healing around a titanium implant.

Ueng and coworkers, using a mechanism by which experimental animals (rabbits) could be exposed to cigarette smoke, reported that intermittent cigarette smoke exposure delayed mineralization during the bone healing process of distraction osteogenesis. In the present investigation, a similar device was used to expose the animals to cigarette smoke by changing the dimensions of the acrylic resin box (45×25×20 cm³) so as to allow the inclusion of 5 animals (rats) each time. In the present study, the amount of cigarettes used at the time of each exposure (ie, 10 cigarettes/exposure) was determined by pilot studies, which had demonstrated that this was the highest volume of cigarette smoke that the animals could support for 8 minutes, 3 times a day, over 60 days. Nevertheless, some animals (4 rats) demonstrated greater sensitivity to such volumes of smoke and died before completing the experimental period. Using a similar protocol (rats in an exposure chamber and 10 cigarettes/exposure), Cendon-Filha reported lung emphysema in the animals after 2 years of daily exposure. Therefore, it was believed that the volume of smoke exposure to which each animal was submitted may have closely assimilated a heavy smoker, ie, an individual who smokes more than 15 cigarettes daily.

Bain and Moy first reported the negative effect of smoking on the success rate of osseointegrated implants. The smokers’ failure rate was 11.28% (44/390), while the nonsmokers’ failure rate was significantly lower, at 4.76% (86/1,804). This observation was later confirmed in different populations using different implant systems. De Bruyn and Collaert described the effect of smoking on early implant failure, ie, before functional loading with fixed prosthetic restorations. The failure rate before loading was 9% in smokers versus 1% in nonsmokers; this difference was statistically significant. The authors concluded that smoking is a significant factor in the failure of implants prior to functional loading.

Gorman and coworkers analyzed more than 2,000 implants regarding their survival at second-stage surgery and concluded that smoking is detrimental to implant success. Lindquist and associates showed that smoking was the most important factor of those correlated with increased peri-implant bone loss. Lambert and colleagues reported that after 3 years, endosseous implants placed in smokers were almost 1.5 times more likely to fail than in nonsmokers (2.9% difference), but both groups demonstrated a high success rate (94%
investigated. And/or before implant placement remains to be
mals were exposed for a longer period of time.
compounds of the cigarette smoke for many years.
by humans, ie, the bone tissue is exposed to the
In addition, cigarette smoke is inhaled chronically
may not entirely reproduce the events in humans.
CONCLUSION
Within the limits of the present study, it was con-
cluded that although cigarette smoke exposure may
not seriously affect cortical bone, it may jeopardize
bone quality around titanium implants in the can-
celous bone area, as seen in this exclusively histo-
logic investigation.
ACKNOWLEDGMENT
The authors greatly appreciate the assistance of AS Technology,
which supplied the implants.
REFERENCES
1. Brånemark P-I. Osseointegration and its experimental back-
2. Lindquist LW, Carlson GE. Long-term effects on chewing
with mandibular fixed prostheses on osseointegrated
3. Albrektsson T, Zarb GA, Worthington P, Ericsson AR. The
long-term efficacy of currently used dental implants: A
review and proposed criteria for success. Int J Oral Maxillo-
4. Zarb GA, Schmitt A. The longitudinal clinical effectiveness
of osseointegrated dental implants: The Toronto study. Part
5. Esposito M, Hirsch J-M, Lekholm U, Thomsen P. Biological
factors contributing to failures of osseointegrated oral
implants (II). Etiopathogenesis. Eur J Oral Sci 1998;106:
721–764.
6. Meechan JG, Macgregor ID, Rogers SN, Hobson RS, Bate
JP, Deninison M. The effect of smoking on immediate post-
extraction socket filling with blood and the incidence of
changed smoking habits on marginal alveolar bone loss. A
8. Holm G. Smoking as an additional risk for tooth loss. J
9. Haber J, Wattles J, Crowley M, Mandrell R, Joshipura K,
Kent RL. Evidence for cigarette smoking as a major risk for
10. Bain CA, Moy PK. The influence of smoking on bone qual-
ity and implant failure [abstract]. Int J Oral Maxillofac
Implants 1994;9:123.
11. Weyant RJ. Characteristics associated with the loss and peri-
implant tissue health of endosseous dental implants. Int J
12. Bain CA, Moy PK. The association between the failure of
dental implants and cigarette smoking. Int J Oral Maxillo-
fac Implants 1993;8:609–615.
The effect of smoking on implant survival at second-stage
of smoking on peri-implant tissue: A retrospective
16. Lindquist LW, Carlson GE, Jemt T. Association between
marginal bone loss around osseointegrated mandibular
implants and smoking habits: A 10-year follow-up study. J
17. Lambert PM, Morris HF, Ochi S. The influence of smoking
on 3-year clinical success of osseointegrated dental implants.
Ann Periodontol 2000;5:79–89.
18. Stefani CM, Nogueira-Filho GR, Sallum EA, Toledo S, Sal-
llum AW, Nociti FH Jr. Influence of nicotine administration
on different implant surfaces: A histometric study in rabbits.
J Periodontol (in press).
19. Donath K, Breuner GA. A method for the study of uncalcifi-
ced bones and teeth with attached soft tissue. J Oral
influence of nicotine on the bone loss rate in ligature-
duced periodontitis. A histometric study in rats. J Perio-


