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

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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*

For well over a decade titanium endosseous implants have been used increasingly often in various edentulous situations.[1–4] However, some local and systemic conditions may impair bone healing or may interfere with the maintenance of osseointegration.[5] It is well-recognized that cigarette smoking is associated with impaired wound healing after surgical treatment in the oral cavity,[6] reduced bone height,[7] an increased rate of bone loss,[8] increased resorption of the alveolar ridge,[7] and a higher incidence of periodontitis[9] and type IV bone.[10] In addition, smoking has been found to be an important factor in peri-implant soft tissue changes.[11]

Smoking has also been one of the factors often discussed in relation to implant failure. Bain and Moy[12] assessed the various factors predisposing to implant failure in a group of 540 patients who had received 2,194 implants. They found that smoking was by far the most significant factor: failure rates were 4.76% in nonsmokers and 11.28% in smokers. In a later study, De Bruyn and Collaert[13] compared implant failures before loading in the maxillae of smokers and nonsmokers. They found that at least 1 failure was detected in 1 in 3 smokers, compared with only 1 in 25 nonsmokers (9% and 1%, respectively). Gorman and coworkers[14] evaluated the relationship between smoking and failure rates of dental implants at second-stage surgery and suggested that smoking is detrimental to implant success. Haas and associates[15] have also suggested that smokers suffer detrimental effects around successfully integrated

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maxillary implants. Lindquist and colleagues[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[5] reviewed the literature regarding factors associated with the loss of oral implants and concluded that a smoking habit was one of the factors associated with biologic failures of implants. Recently, Lambert and associates[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[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 (Pentabiótico, 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 $45 \times 25 \times 20$-cm^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,[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).

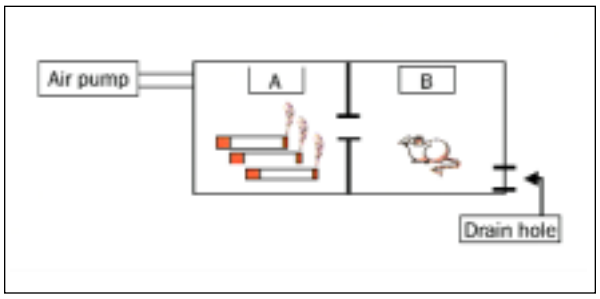

Fig 1 Schematic illustration of the cigarette smoke exposure device. The acrylic resin chamber was composed of 2 subchambers: the cigarette compartment (A) and the animal compartment (B).

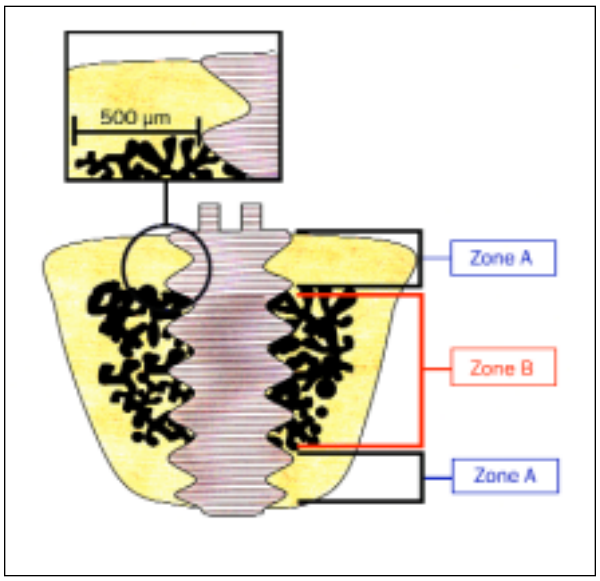

Fig 2 Schematic illustration of the histometric parameters evaluated.

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 associates[17] 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,

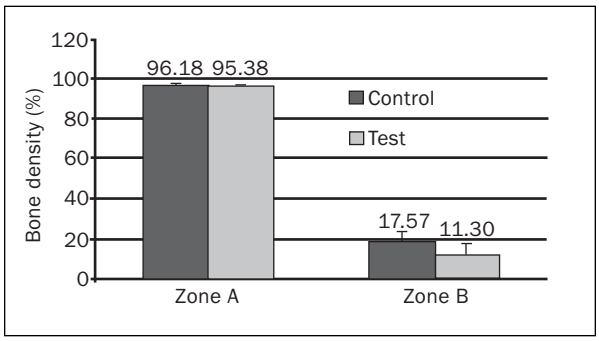

Fig 3 Mean and standard deviation (%) of the bone density around the implants for groups 1 and 2 at zones A and B.

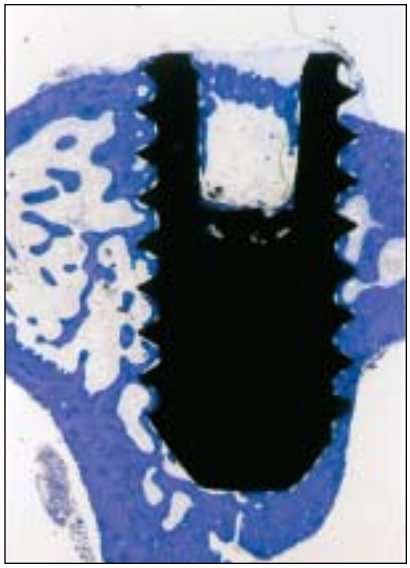

Fig 4 Histologic aspect of an implant placed in a group 1 animal (toluidine blue; original magnification ×6.25).

Fig 5 Histologic aspect of an implant placed in a group 2 animal (toluidine blue; original magnification ×6.25).

the present study was proposed to investigate whether cigarette smoke inhalation would interfere with the bone healing around a titanium implant.

Ueng and coworkers,[25,26] 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 cm^3) 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[27] 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[12] 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[13] 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[14] 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[16] showed that smoking was the most important factor of those correlated with increased periimplant bone loss. Lambert and colleagues[17] 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%

versus 91.1% for nonsmokers and smokers, respectively). The difference between smokers and nonsmokers reported by Lambert and associates[17] (2.9%) is less than half that reported by Bain and Moy[12] (6.52%). A possible reason for this discrepancy lies in the fact that Bain and Moy studied 100% machined implants, while Lambert used mostly textured implants (HA-coated); this signifies that the failure rate may be influenced by implant design and surface.

At a histologic level, the present study showed that intermittent cigarette smoke inhalation may influence bone density in the cancellous bone area around titanium implants, although no significant effect was observed in cortical bone. The clinical relevance of such an observation requires further investigation, although it seems to support the high success rates observed for smokers in the study of Lambert and coworkers.[17]

While in the present study, the animals were submitted to all of the compounds of the cigarette smoke, as are humans, caution must be used in extrapolating the results. First, the local effect of cigarette consumption was not a factor in the present study. Second, the implants were not loaded, and consequently, on a long-term basis, the real implications of lower bone density in the cancellous bone region after loading the implants for a period of time could not be projected. Finally, despite the fact that rats have been used as a model to test some hypotheses regarding titanium implants,[28–35] this may not entirely reproduce the events in humans. In addition, cigarette smoke is inhaled chronically by humans, ie, the bone tissue is exposed to the compounds of the cigarette smoke for many years. Whether different results would be observed if animals were exposed for a longer period of time and/or before implant placement remains to be investigated.

CONCLUSION

Within the limits of the present study, it was concluded that although cigarette smoke exposure may not seriously affect cortical bone, it may jeopardize bone quality around titanium implants in the cancellous bone area, as seen in this exclusively histologic investigation.

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