Bone Density Around Titanium Implants May Benefit from Smoking Cessation: A Histologic Study in Rats

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Purpose: *This study tested the hypothesis that interruption of cigarette smoke inhalation (CSI) would reverse its impact on bone quality around implants.* ***Materials and Methods:*** *Sixty-nine rats were assigned to 1 of 4 groups. Group 1 (n = 16) was the control group; group 2 experienced CSI for the duration of the study (150 days); group 3 experienced CSI for 83 days prior to implant placement, until 7 days prior to implant placement, when CSI ceased; and for group 4, CSI exposure was temporarily halted from 7 days before implantation to 21 days afterward. Bone density (the proportion of mineralized bone in a 500-µm-wide zone lateral to the implant) was calculated for each specimen (mean ± SD).* ***Results:*** *In the cortical bone, a slight difference in bone density was noted between the groups (97.66% ± 3.69% for group 1, 98.30% ± 0.95% for group 2, 98.83% ± 0.73% for group 3, and 98.11% ± 1.14 for group 4;* $P > .05$*). In contrast, continuous exposure to cigarette smoke (group 2) significantly decreased density in the cancellous bone in comparison to the other groups (25.69% ± 9.41% for group 1, 18.08% ± 6.0% for group 2, 25.46% ± 5.42 for group 3, and 26.20% ± 6.77% for group 4;* $P < .05$*), with no significant differences between groups 1, 3, and 4 (*$P > .05$*).* ***Discussion:*** *The results support the concept that the effects of cigarette consumption on dental implants may be reversible, and therefore suggest that smokers may realize satisfactory outcomes if they cease smoking, even temporarily.* ***Conclusion:*** *In conclusion, smoking may affect bone quality around titanium implants in cancellous bone, and cessation could result in a return toward to the levels of the control group.* INT J ORAL MAXILLOFAC IMPLANTS 2005;20:713–719

Key words: *dental implants, osseointegration, smoking, smoking cessation*

Over the past 20 years endosseous titanium implants placed under various modifications of the original Brånemark protocol have proven to be among the most predictable treatments in oral health care. Success rates in excess of 95% up to 15 years and beyond compare favorably with other methods of tooth replacement. Quality of life assessments comparing implant-supported prostheses with removable partial and complete dentures have shown the implant-retained prosthesis to be a highly satisfactory method of tooth replacement.[1,2] However, some systemic conditions have been correlated with higher rates of failure.[3] Smoking is one of the factors often discussed in relation to implant failure.[2,4–8] It is well recognized that cigarette smoking is associated with impaired wound healing after surgical treatment in the oral cavity,[9] reduced bone height,[10] increased bone loss rate,[11–13] increased resorption of the alveolar ridge,[11] and higher incidence of periodontitis[14] and type 4 bone.[15]

Several studies have provided evidence that the impact of tobacco smoking on oral structures may be reversible. In a 10-year study, Bolin and associates[10] showed that the progression of bone loss was significantly retarded in individuals who had given up smok-

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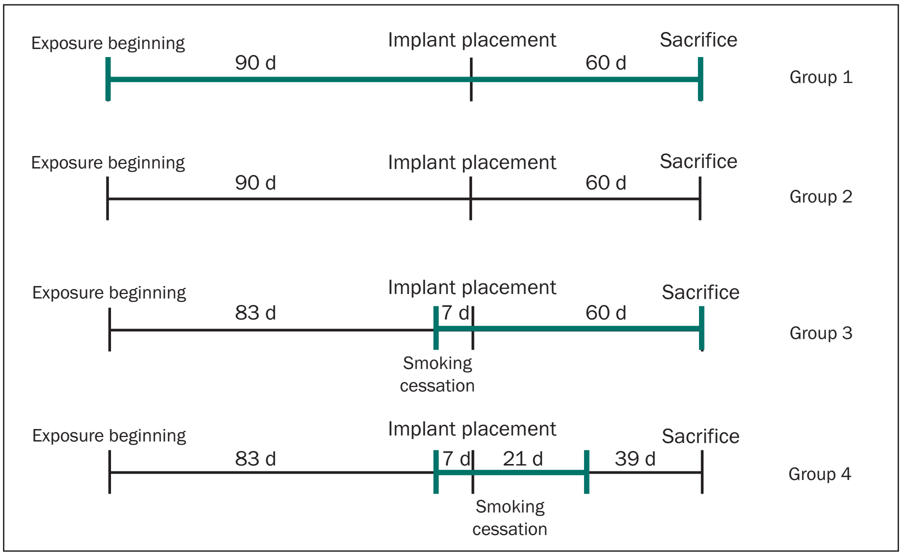


Fig 1 Schematic illustration of the experimental design. Green lines indicate smoking cessation.

ing. Liede and colleagues[16] compared periodontal status, salivary proteolytic activity, and oral mucosal status in individuals who had quit smoking to those of regular smokers, and found that periodontal status and mucosal health were better in those who had quit smoking. Gingival microcirculation has also been shown to recover its normal blood flow in the early stages of smoking cessation,[17] and changes in the inflammatory response of the periodontium can also be reversible upon smoking cessation.[18] Additionally, former smokers have been reported to present periodontal bone height reduction rates similar to nonsmokers,[13] and therefore to lose significantly less marginal bone in a period over 20 years than self-described smokers during the same period.[12]

Unfortunately, there is much more evidence of the detrimental effect of smoking on implant outcomes than there is on the potential benefits of stopping smoking. In 1 study, Bain[19] clinically examined a cessation protocol in which potential implant patients who smoked were encouraged to stop for 1 week before and 8 weeks after implant placement. In a prospective study of 223 consecutive Bränemark System implants placed in 78 patients, the authors found no difference in failure rate between nonsmoking controls and the smokers who quit, whereas a significant difference was noted between the continuing smokers and smokers who followed the cessation protocol.

Because only very limited studies are available in regard to the clinical relevance of this subject, the present study aimed to provide additional information on whether smoking cessation during the healing phase would affect bone density around titanium implants, as well as whether complete rather than temporary cessation would be required to achieve bone quality similar to that found in the nonsmoking group.

MATERIALS AND METHODS

Animals

Sixty-nine male Wistar rats (300 to 400 g) were included 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 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 obtained by intramuscular administration of ketamine (0.5 mL/kg). Skin was cleansed with iodine surgical soap. An incision approximately 1 cm in length was made, and the tibiae surfaces were surgically exposed unilaterally by blunt dissection. Under profuse saline irrigation, bicortical implant beds were drilled at a rotary speed not exceeding 1,500 rpm. One screw-type commercially available pure titanium implant, of 4.0 mm in length and 2.2 mm in diameter, was placed until the screw thread had been completely introduced into the bone cortex. Finally, soft tissues were replaced and sutured over the implant (cover screw was used). Postoperatively, the animals received an antibiotic (1mL/kg;-Pentabiótico, Wyeth-Whitehall, São Paulo, SP, Brazil) given through a single intramuscular injection.

Experimental Design

Ninety days before implant surgery, the animals were randomly assigned to 1 of the following groups (Fig 1):

- **Group 1:** The control group (n = 16)
- **Group 2:** Intermittent cigarette smoke inhalation (CSI) (n = 17)
- **Group 3:** CSI for 83 days prior to implant placement; cessation of CSI 7 days before implant placement (n = 16)
- **Group 4:** Cessation of CSI 7 days before implant placement; resumption 21 days after implant placement (n = 20)

Animals that died before sacrifice were withdrawn from the study; thus, the groups contained slightly different numbers of animals.

The animals of groups 2, 3, and 4 were intermittently housed in an animal cigarette smoke exposure chamber as previously described.[20,21] Briefly, the device consisted of a 45 × 25 × 20 cm clear acrylic resin chamber, an air pump, and 2 inflow/outflow tubes (Fig 2). Five animals were housed in the chamber at a time, and the cigarette smoke of 10 cigarettes, containing 1.3 mg of nicotine, 16.5 mg of tar, and 15.2 mg of carbon monoxide each, was pumped into the chamber. Thus, the animals were forced to breathe the cigarette smoke that contaminated the air for 8 minutes, 3 times daily until they were sacrificed 60 days after implant placement. The animals of group 1 were not exposed to the cigarette smoke at any time. The serum levels of nicotine and cotinine obtained using this model have been previously reported.[22]

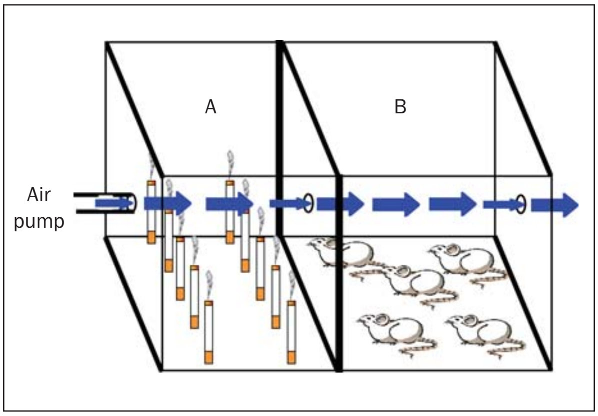


Fig 2 Schematic illustration of the cigarette smoke exposure device. The acrylic resin chamber was composed of 2 subchambers: the cigarette compartment (A) and the animal compartment (B).

Histometric Procedure

Sixty days after implant placement, the animals were sacrificed; the tibiae were removed and fixed in 4% neutral formalin for 48 hours. Undecalcified sections were prepared as previously described;[23] ie, the blocks were dehydrated by using an ascending series of ethanol (60% to 100%) and embedded in glycolmethacrylate resin (Technovit 7200; Heraeus Kulzer, Wehrheim, Germany). Subsequently, the sections (20 to 30 µm) were obtained and stained by using toluidine blue 1% staining. Bone density (ie, the proportion of mineralized bone in a 500-µm-wide zone lateral to the implant) was obtained (Image-Pro; Media Cybernetics, Silver Springs, MD) bilaterally in the cortical (zone A) and cancellous bone (zone B) areas by a blinded examiner.

Statistical Analysis

The data from zones A and B (cortical and cancellous bone, respectively) were averaged separately. Results are presented as means ± SDs. The null hypothesis, ie, bone density was neither influenced by CSI nor by the cessation protocols, was tested by an intergroup analysis using the parametric 1-way analysis of variance (ANOVA) test (alpha = .05), regarding zones A and B separately (group 1 versus group 2 versus group 3 versus group 4). If statistical difference was detected by the 1-way ANOVA test, a pair-wise multiple comparison procedure was performed by the Tukey test (alpha = .05) to detect the differences between the groups.

RESULTS

Histometric Analysis

Although a slight difference was observed, the intergroup analysis (1-way ANOVA) did not reveal significant differences between the groups with respect to the bone density for zone A. The bone densities were determined to be 97.66% ± 3.69%, 98.30% ± 0.95%, 98.11% ± 1.14%, and 98.83% ± 0.73%, for groups 1 to 4, respectively ($P = .38$)

In contrast, the intergroup analysis (1-way ANOVA) showed a significant difference among the groups for zone B (25.69% ± 9.41%, 18.08% ± 6.07%, 25.46% ± 5.42%, and 26.20% ± 6.77%; groups 1 to 4, respectively; $P = .002$). The pair-wise comparison (Tukey test) indicated that continuous exposure to cigarette smoke (group 2) significantly decreased bone density in the cancellous bone ($P < .05$) in comparison with the other groups. Moreover, the pair-wise comparison (Tukey test) showed no significant differences between groups 1, 3, and 4 ($P > .05$) (Fig 3). Figs 4a to 4d illustrate the histologic results for the experimental groups.

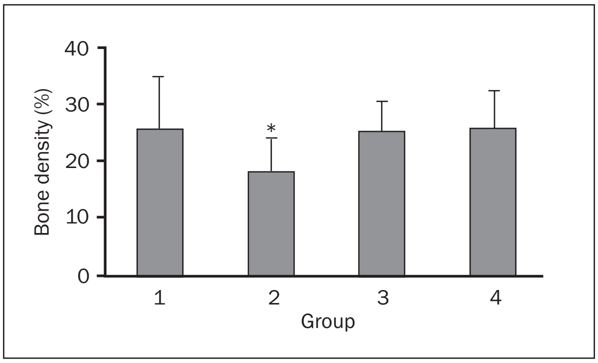
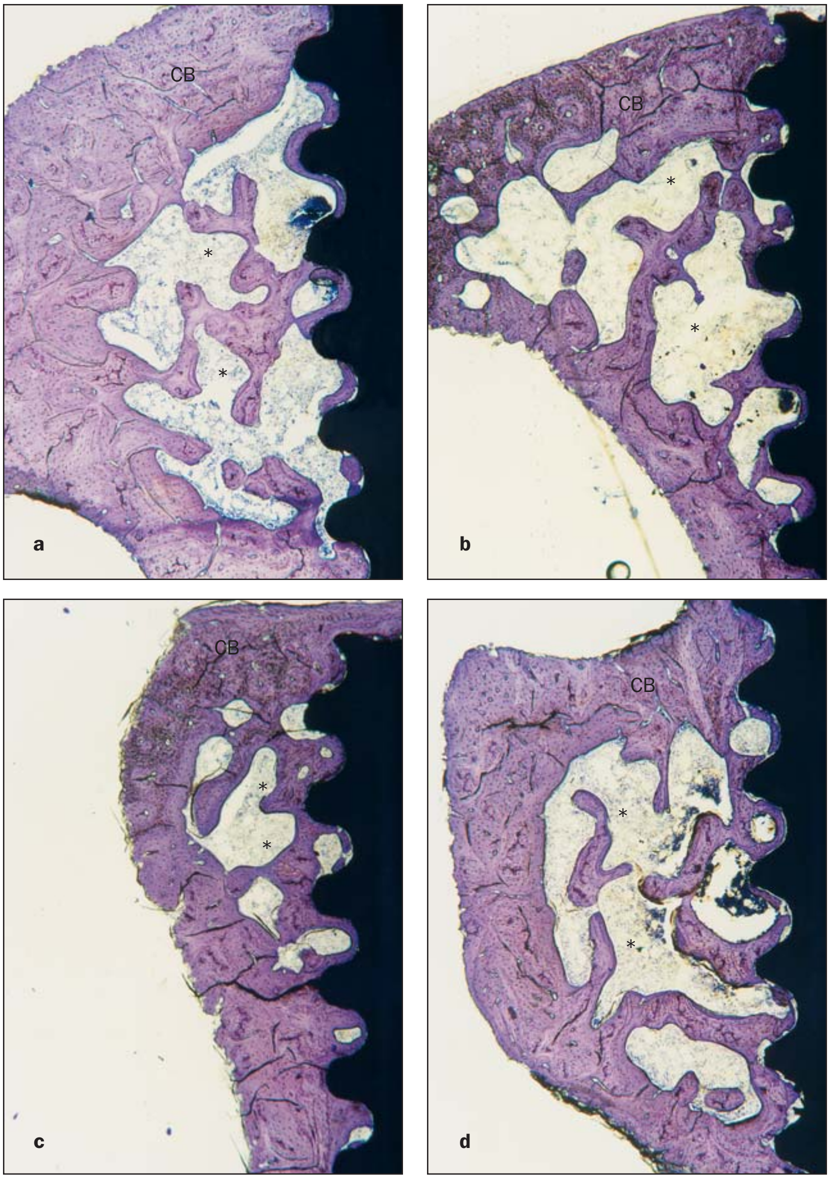


Fig 3 Means and standard deviations (%) of bone density in zone B around the implants in groups 1, 2, 3, and 4.

*Intergroup analysis showed lower BD for group 2 to be significantly greater than for groups 1, 3, and 4. (1-way ANOVA; *P*=.0002).



Figs 4a to 4d Histologic specimens from *(a)* group 1, *(b)* group 2, *(c)* group 3, and *(d)* group 4 60 days after implant placement. Note that the results were more intense in zone B (*) versus zone A (toluidine blue; original magnification ×6.25).

DISCUSSION

The present investigation histologically evaluated the impact of intermittent CSI on bone healing around titanium implants placed in the tibiae of rats (specifically, on the proportion of mineralized tissue found in a 500-μm-wide zone lateral to the implant surface). The authors also sought to determine whether 2 different CSI cessation protocols would prevent the impact of CSI on bone. Data analysis demonstrated that the cortical bone (zone A) was not significantly affected either by CSI or by any of the cessation protocols. On the other hand, CSI significantly reduced BD in the cancellous bone (zone B). Additionally, definitive or temporary cessation protocols were both able to reverse the effect of CSI on BD.

During the early phase of implant procedure development, implant failure was generally attributed to poor surgical technique (infection, overheating of bone, overinstrumentation), poor prosthetic design or management, or patient-related factors (limited available bone, poor oral hygiene, occlusal overload). These findings were largely based on clinical observation, extrapolation from failures of tooth-supported prostheses, and dogma. Jones and Triplett[24] evaluated the influence of smoking on wound healing in patients undergoing intraoral bone grafting and simultaneous implant placement and may have been among the first to implicate smoking as a potential risk factor. Smoking is now one of the factors often discussed in relation to decreased success rates of dental implants. Bain and Moy[5] assessed predisposition 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[4] 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 associates[6] evaluated the relationship between smoking and the failure rates of dental implants at second-stage surgery. They suggested that smoking is detrimental to implant success. Haas and colleagues[7] have also reported that smokers suffer harmful effects around successfully integrated maxillary implants. Lindquist and coworkers[2] 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 colleagues[3] reviewed the literature regarding factors associated with the loss of oral implants and concluded that a smoking habit was 1 of the factors associated with biologic failure of the implants.

Recently, Lambert and associates[8] 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, a series of studies has tried to document, at a histologic level, the influence of cigarette consumption and/or its compounds on bone healing around titanium implants. Stefani and coworkers[25] investigated the effect of nicotine administration on the osseointegration process around dental implants. Nicotine was observed to have a slight negative effect on bone-to-implant contact around implants with machined surfaces, although this difference was not statistically significant. Nociti and colleagues[21] demonstrated that cigarette smoke exposure may jeopardize bone quality around titanium implants in the cancellous bone area. César-Neto and associates[22] investigated the impact of 2 conditions, nicotine administration and cigarette smoke inhalation, on the healing around implants, and concluded that the negative impact of smoking on implant outcomes may be related to more than 1 substance present in the cigarette smoke and that nicotine does seem to be one of the substances involved, especially in cancellous bone.

Reversibility of the effects of cigarette consumption has been studied both in medicine and dentistry. For lung disease, 1 of the most frequent pathologies associated with cigarette consumption, a former smoker is considered to run the same risk as a nonsmoker 15 years after smoking cessation.[26] In addition, it has been shown that a current smoking habit had a stronger effect on mean total white blood cell counts than cumulative exposure.[27] The effects of smoking on white blood cell counts demonstrated an almost immediate reduction after smoking cessation.

In dentistry, smoking cessation has also been shown to positively impact periodontal risk. In vitro studies[28,29] have suggested that the cytotoxic effect of cigarette compounds (ie, nicotine, acrolein, and acetaldehyde) on periodontal cells is reversible. The relative risk was reported to be 3.97 for smokers and 1.68 for former smokers.[30] In addition, among former smokers, the risk decreased with the number of years since quitting (to 3.22 after 2 years and to 1.15 after 11 years). In a prospective study over 20 years,[12] 507 individuals were radiographically evaluated, and the results showed that those who stopped smoking during the experimental period lost significantly less marginal bone when compared to current smokers. Another longitudinal study[31] evaluated the changes on the periodontal status of 101 patients over 10 years. Clinically, the frequency of diseased sites found increased in smokers, while remaining steady or decreasing in former smokers and nonsmokers. Radiographically, increased bone loss for current smokers was noted in comparision to former smokers and nonsmokers. No significant differences were observed between former smokers and nonsmokers.

Moreover, smoking cessation has also been reported to be beneficial in regard to periodontal treatment outcome.

Grossi and colleagues[32] demonstrated that former smokers and nonsmokers presented significantly more healing and reduction of *Bacteroides forsythus* and *Porphyromonas gingivalis* than current smokers. Therefore, the results of the present study appear to agree with previous reports showing a reversible condition promoted by cigarette consumption. In the implant field, very limited information is available with respect to the reversibility of the effects of smoking on implant outcomes. Bain[19] was the first to report that a smoking cessation protocol would improve success rates for osseointegration in smokers. Thus, the results of the present study support the concept that the effects of cigarette consumption on dental implants may be reversible, and therefore suggest that smokers may realize satisfactory outcomes if they cease smoking, even temporarily.

Misclassification of smoking status has been a concern in the literature[33] and is considered a confounder in epidemiologic studies. Inaccurate reports may occur for many reasons, such as individual metabolism, frequency of inhalation, depth of inhalation, capacity for dilution with room air, amount of cigarette stub left, and cigarette brand.[28] Biochemical validation of smoking status seems to be useful to minimize the influence of confounders in clinical studies, mainly for the determination of light, regular, and heavy smokers. In animal studies such confounders may be more accurately controlled. It has been previously reported[22] that the CSI regimen used in the present study promoted cotinine serum levels closely correlated with smokers that smoke 10 to 20 cigarettes/day.[34] However, future comparisons with humans should be treated with caution because of differences in the metabolism of nicotine between humans and rats and the frequency of smoke administration used in this study.

CONCLUSION

Within the limits of the present investigation, it can be concluded that the effects of smoking on bone around titanium implants may be reversible and that a temporary smoking cessation protocol may be as beneficial as a definitive one.

ACKNOWLEDGMENTS

The authors greatly appreciated the assistance of AS Technology in supplying the implants. Dr César-Neto was supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP, Brazil, 02/08554-0). Dr Nociti was supported by the National Council of Research (304464/03-1, CNPq, Brazil).

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