Bone Filling Around Titanium Implants May Benefit From Smoking Cessation: A Histologic Study in Rats

João B. César-Neto,* Bruno B. Benatti,* Enilson A. Sallum,* Antonio W. Sallum,* and Francisco H. Nociti Jr.*

**Background:** Although a harmful effect of smoking on titanium implants has been documented, only a few studies have investigated the benefits of smoking cessation. Thus, this study histologically investigated whether smoking cessation influences bone healing around titanium implants and comparatively evaluated temporary versus complete cessation protocols.

**Methods:** Sixty-six male Wistar rats were randomly assigned to one of four groups: control (N = 16); CSI, intermittent cigarette smoke inhalation (CSI) throughout the entire 150-day experiment (N = 17); P-CSI, CSI 83 days prior to implant placement (N = 17); or R-CSI, CSI for 83 days, cessation 7 days before and 21 weeks after implant placement, and return to CSI for 39 days (N = 16). The animals were sacrificed and the degree of bone-to-implant contact (BIC) and the bone area (BA) within the limits of the implant threads obtained for cortical (zone A) and cancellous bone (zone B).

**Results:** Intergroup analysis (Kruskal-Wallis test, α = 0.05) demonstrated a significant effect of CSI on BA (P < 0.05) and that temporary or complete CSI cessation protocols resulted in values similar to those of the control group (P > 0.05) 88.91% ± 4.29%; 80.66% ± 6.55%; 84.27% ± 6.96%; 85.71% ± 4.7% in zone A, and 51.28% ± 6.49%; 38.69% ± 10.78%; 48.87% ± 8.47%; 49.47% ± 8.04%; in zone B for groups CTRL, CSI, P-CSI, and R-CSI, respectively.

**Conclusion:** Within the limits of the present investigation, temporary and complete CSI cessation reversed the effect of smoke exposure on bone healing around titanium implants in both cortical and cancellous bone. *J Periodontol* 2005;76:1476-1481.

**KEY WORDS**

Animal studies; bone and bones; dental implants; osseointegration; smoking/adverse effects; smoking cessation; titanium; wound healing.
response of the periodontium may also be reversible on quitting smoking.\textsuperscript{20}

Although highly recommended in the dental implant field, the most obvious remedy to the adverse effects of cigarette consumption, smoking cessation, has not been extensively explored. In a prospective study of 223 consecutive implants placed in 78 patients, Bain\textsuperscript{21} found no difference in the failure rate between non-smoking controls and the smokers who quit, whereas a significant difference was noted between the continuing smokers and smokers who followed the cessation protocol. Therefore, some evidence exists to suggest that smoking cessation should be considered when recommending dental implants to smokers. Based on the clinical relevance of this subject, and the limited number of available studies, the present study aimed to histologically investigate in rats whether smoking cessation during the healing phase may affect bone formation around titanium implants and whether a complete rather than a temporary cessation protocol would be required in order to achieve bone profiles similar to the non-smoking group.

MATERIALS AND METHODS

Animals

Sixty-six 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 \textit{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 cleaned with iodine surgical soap. An incision of approximately 1 cm in length was made and the bone surface of the tibia surgically exposed by blunt dissection. Under profuse saline irrigation bicortical implant beds were drilled at a rotary speed not exceeding 1,500 rpm. One screw-shaped commercially available pure titanium implant, 4.0 mm in length and 2.2 mm in diameter, was placed until the screw thread was completely introduced into the bone cortex; 2.2 mm in diameter, was placed until the screw thread was complete. Under profuse saline irrigation bicortical sections. After sacrifice, the tibiae were removed and fixed in 4% neutral formalin for 48 hours. Undecalcified sections were prepared as previously described;\textsuperscript{22} i.e., the blocks were dehydrated by using an ascending series of ethanol (60% to 100%) and embedded in glycol-methacrylate.\textsuperscript{3} Subsequently, sections (20 to 30 \textmu m) were obtained and stained by 1% toluidine blue staining. The percentage of bone-to-implant contact (BIC) and bone area (BA) within the threads of the implants were obtained\textsuperscript{8} by a masked examiner. The data were arranged separately in cortical (zone A) and cancellous bone (zone B) areas, as previously described.\textsuperscript{10,11}

Statistical Analysis

Data from zones A and B were separately averaged. The null hypothesis: i.e., BIC and BA were neither influenced by CSI nor by the cessation protocols in zones A and B, was tested by an intergroup analysis using the non-parametric Kruskal-Wallis test ($\alpha=0.05$) (CRTL versus CSI versus P-CSI versus R-CSI).

Pairwise multiple comparisons were carried out by Dunn’s test ($\alpha=0.05$) when the Kruskal-Wallis test showed significant differences.

RESULTS

Histometric Results

Although a slight difference was observed, statistical analysis did not reveal significant differences among the groups with respect to BIC in either cortical or cancellous bone ($P>0.05$) (55.34\%\pm14.57\%\; 47.84\%\pm11.36\%\; 51.09\%\pm11.97\%; 47.77\%\pm12.43\%\; in zone A and 14.57\%\pm10.78\%; 33.21\%\pm12.51\%; 39.67\%\pm11.96\%; 37.46\%\pm10.28\%\ in zone B; for control, CSI, P-CSI, and R-CSI groups, respectively). In contrast,
confirming previous findings by our group, continuous exposure to cigarette smoke (CSI) significantly affected BA when compared to the control group in both cortical and cancellous bone. In addition, no differences were observed with respect to BA between groups 3 and 4 (cessation groups) and the control group ($P > 0.05$) (88.91% ± 4.29%; 80.66% ± 6.55%; 84.27% ± 6.96%; 85.71% ± 4.7% in zone A and 51.28% ± 6.49%; 38.69% ± 10.78%; 48.87% ± 8.47%; 49.47% ± 8.04% in zone B for control, CSI, P-CSI, and R-CSI groups, respectively). Figures 1 to 3 histologically and graphically illustrate the results for each experimental group.

**DISCUSSION**

The present investigation histologically evaluated whether smoking cessation affects bone healing around titanium implants placed in animals intermittently exposed to cigarette smoke and compared the possible benefits that temporary versus complete cessation protocols would exert on the newly-formed bone. The results confirmed that CSI might significantly affect bone volume in the cortical and cancellous bone around the implant. Additionally, data analysis demonstrated that both temporary and complete cessation protocols positively affect newly-formed bone, resulting in BA values similar to those of the non-smoking control group.

During the early period of implant procedure development, implant failure was generally attributed to poor surgical technique (infection, overheating of bone, or overinstrumentation), poor prosthetic design or management, or patient-related factors (limited available bone, poor oral hygiene, or occlusal overload). These findings were largely based on clinical observation, extrapolation from failures in tooth-supported prostheses, and dogma. However, evidence later emerged correlating systemic conditions with higher failure rates. Esposito et al. reviewed the literature regarding factors associated with the loss of oral implants and concluded that smoking was one of the strongest factors associated with biological failures of implants. Several other clinical studies have provided additional evidence that smokers present higher rates of implant failure, as well as suffer detrimental effects around successfully integrated implants. More recently, a series of studies have suggested, at the histologic level, that cigarette smoke and its compounds may affect bone volume around titanium implants, and therefore take part in the process by which smoking negatively affect implant outcome. Stefani et al. observed a slight negative effect of nicotine on the bone-to-implant contact around implants.
with machined surfaces, although this difference was not statistically significant. Nociti et al.\textsuperscript{10} demonstrated that cigarette smoke exposure may jeopardize bone quality around titanium implants in the cancellous bone area. Finally, César-Neto et al.\textsuperscript{12} comparatively investigated the impact of nicotine administration or cigarette smoke inhalation, on the healing around implants and found that the negative impact of smoking on implant outcomes may be related to more than one molecule present in the cigarette smoke and nicotine seems to partially contribute to this effect. Therefore, the findings of the present study that CSI affects bone around titanium implants reproduce and confirm previous reports.

Very limited information is available in the implant field regarding reversing the effects of smoking on implant outcome. Bain\textsuperscript{21} was the first to report that a smoking cessation protocol would improve implant success rates in smokers who follow it. The beneficial effects of smoking cessation also have been observed in medicine with respect to several tissues. A meta-analysis study demonstrated that current smokers presented a significantly reduced bone mass when compared to former and never smokers and that former smokers presented bone mass that is intermediate or similar to never smokers.\textsuperscript{14} They additionally reported that smoking has an independent, dose-dependent effect on bone loss, which increases fracture risk, and that smoking cessation may present a beneficial effect.\textsuperscript{14} Regarding bone healing, it was observed that patients who quit smoking for periods longer than 6 months, after instrumented spinal fusion presented nonunion rates similar to non-smokers.\textsuperscript{23} For lung disease, one of the most frequent cigarette-related pathologies, a former smoker is considered to run the same risk as a non-smoker 15 years after smoking cessation.\textsuperscript{24} However, it has been reported that cigarette consumption negatively affects white blood cell counts and such an effect is promptly reversed after smoking cessation.\textsuperscript{25} The reversibility of smoking effects has also been investigated in dentistry. In vitro studies observed a reversible condition promoted by cigarette compounds (i.e., nicotine, acrolein, and acetaldehyde) on periodontal cells.\textsuperscript{26,27} Smoking cessation also exerted a beneficial effect on periodontal risk, which decreased with the number of years since quitting.\textsuperscript{28} Longitudinal studies showed that patients who stopped smoking lost significantly less marginal bone than current smokers.\textsuperscript{18,29} Additionally, smoking cessation has also been considered beneficial to periodontal therapy, presenting more healing and reduction of \textit{Bacteroides forsythus} and \textit{Phorphyromonas gingivalis}.\textsuperscript{30} The results of the present investigation are in agreement with the studies that showed a reversible condition promoted by cigarette consumption and support the clinical concept that the effect of cigarette consumption on dental implants may be reversible.

Misclassification of smoking status has been a concern in the literature\textsuperscript{31} and is considered a confounder in clinical studies. Therefore, biochemical validation of smoking status seems to be useful in order to minimize the influence of such confounders. In this sense, a previous study\textsuperscript{12} has reported that a CSI regimen...
similar to the one used in the present study resulted in cotinine serum levels closely correlated with smokers who smoke between 10 to 20 cigarettes/day, as reported by Gonzalez et al. 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. Moreover, additional studies should be considered to investigate whether the decreased bone volume around implants, promoted by cigarette consumption, is clinically relevant. If such a relationship is confirmed, recommendations regarding smoking cessation should be considered as part of the approach to implant patients. Within the limits of the present investigation, both temporary and complete CSI cessation protocols positively affected bone healing around titanium implants, in both cortical and cancellous bone. Therefore, a short-term cessation protocol during the healing phase may result in bone filling of the threads similar to non-exposed animals. However, further studies should be considered in order to determine whether the return to a smoking condition may affect the bone around the implant on a long-term basis.

ACKNOWLEDGMENTS

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

REFERENCES


