# Influence of Nicotine on Healing Process of Autogenous Bone Block Grafts in the Mandible: A Histomorphometric Study in Rats

Samara Bonfante, MS, DDS[1]/Alvaro Francisco Bosco, MS, DDS, PhD[2]/Danielle Shima Luize, DDS, MS[2]/Juliano Milanezi de Almeida, MS, DDS, PhD[3]/Tania Mary Cestari[4]/Rumio Taga, MS, DDS, PhD[5]

***Purpose:*** *The aim of this study was to perform qualitative and quantitative analyses of the effect of nicotine on autogenous bone block grafts and to describe events in the initial healing phase and the differences in the repair processes between animals exposed to nicotine and controls.* ***Materials and Methods:*** *Forty-eight female Wistar rats were randomly divided into 2 groups, the nicotine group and the saline group. All animals received either nicotine (3 mg/kg) or saline 4 weeks before the surgical procedure and continued to receive nicotine from surgery to sacrifice at 7, 14, or 28 days. The autogenous bone block graft was harvested from the calvaria and stabilized on the external cortical area near the angle of the mandible.* ***Results:*** *The histologic analyses of the nicotine group depicted a delay in osteogenic activity at the bed-graft interface, as well as impairment of the organization of the granulation tissue that developed instead of blood clot. Nicotine-group specimens exhibited less bone neoformation, and the newly formed bone was poorly cellularized and vascularized. The histometric analysis revealed significantly less bone formation in the nicotine group at both 14 days (23.75% ± 6.18% versus 51.31% ± 8.31%) and 28 days (42.44% ± 8.70% versus 73.00% ± 4.99%).* ***Conclusion:*** *Nicotine did jeopardize the early healing process of autogenous bone block grafts in rats but did not prevent it.* Int J Oral Maxillofac Implants 2008;23:437–444

**Key words:** *autograft, bone grafting, nicotine, wound healing*

Tobacco use has been directly associated with a variety of medical conditions and is one of the most preventable causes of disease. Cigarette smoking has been established as one of the most significant risk factors in the onset and progression of periodontal disease.[1,2] Several clinical and epidemiologic studies indicate that cigarette smoking jeopardizes the response to surgical and nonsurgical procedures.[3–9] The precise mechanisms by which tobacco smoke interferes with healing are not completely understood. However, a large number of potentially toxic substances are found in tobacco. Nicotine is not only one of the most important compounds of the particulate phase of tobacco smoke but also the most investigated.[10,11] Several studies in animals have demonstrated that systemic administration of nicotine may reduce revascularization and osteogenesis,[12–16] delay alveolar healing of extraction sockets[17] and bone healing of defects treated by guided tissue regeneration,[18] and enhance bone loss in furcation regions regardless of plaque infection.[19] Clinical studies have shown an association between dental implants in augmented maxillary sinuses and a history of smoking.[20] A higher implant failure rate after rehabilitation of maxillae with and without bone grafts[21–24] has been associated with smoking.

There is limited information regarding the effect of cigarette compounds, such as nicotine, on the initial healing process of autogenous bone block grafts. Thus, the purpose of this study was to perform quali-

[1]Postgraduate Student and PhD Candidate, Dental School of Araçatuba, University of São Paulo, Brazil.
[2]Associate Professor, Dental School of Araçatuba, University of São Paulo, Brazil.
[3]Research Fellow, Dental School of Araçatuba, University of São Paulo, Brazil.
[4]Postgraduate Student and PhD Candidate, Department of Biological Sciences, Bauru Dental School, University of São Paulo, Brazil.
[5]Chairman, Department of Biological Sciences, Bauru Dental School, University of São Paulo, Brazil.

**Correspondence to:** Dr Samara Bonfante, Rua Vivaldo Guimarães no 17-62 Jardim Dona Sarah; CEP:17012-120, Bauru, São Paulo, Brasil. E-mail: sbonfante@uol.com.br Fax: +55 14 32342566.

tative and quantitative analyses of the effect of nicotine on autogenous bone block grafts in rats, including the description of events in the initial healing phase and differences in the repair processes between rats exposed to nicotine and controls.

## MATERIALS AND METHODS

### Animals

Forty-eight Wistar female rats (300 to 400 mg) were included in the study. The animals were kept in plastic cages with access to food and water ad libitum. The present research protocol was approved by the University of São Paulo, Institutional Animal Care and Use Committee (no. 33/03).

### Experimental Design

The animals were randomly assigned into 2 study groups, the nicotine group and the saline group. The nicotine group received subcutaneous injections of nicotine (3 mg/kg) twice daily at 12-hour intervals (n = 24). The saline group received saline under the same conditions. All animals received either nicotine or saline for a period of 4 weeks before surgical procedure of autogenous bone block graft. After surgery, the nicotine or saline regimen continued until the time of sacrifice (7, 14, or 28 days postoperatively).

Nicotine hemisulfate (Sigma Chemical, St Louis, MO) was diluted in saline at the concentration of 5 mg/mL for the nicotine group. Each rat received a volume of diluted solution calculated as 3 mg/kg weight according to the procedure proposed by Okamoto et al.[25]

General anesthesia was obtained by intramuscular administration of xylazine (0.3 mL/100g body weight) plus ketamine (0.7 mL/100 g body weight). The calvaria was used as a donor site, and the angle of the mandible was the recipient area. After anesthesia, the skin of the donor and recipient areas was trichotomized, and a vigorous disinfection was accomplished with 0.2% chlorhexidine digluconate (Colgate-Palmolive, New York, NY).

The bone graft was harvested from the right parietal bone using a surgical trephine with an internal diameter of 3.8 mm (Prudent, Lins, São Paulo, Brazil), with a controlled speed of 800 rpm, under abundant and continuous irrigation with physiologic saline. The bone block was then drilled in its center with a 0.5-mm carbide bur (KG Sorensen, Barueri, São Paulo, Brazil) at low speed and cooled with physiological saline. The recipient area was also drilled with the same carbide bur, allowing a green polyester 5-0 suture thread (Johnson & Johnson/Ethicon, Somerville, NJ) to go through the mandible and the bone block. This procedure allowed placement and stabilization of the graft in close contact with the mandibular bone surface. The wound was sutured in layers, ie, the muscle was sutured with 5-0 polyglactin 910 (Vicryl; Johnson & Johnson/Ethicon), and the skin in the donor and recipient areas was sutured with 4-0 black silk (Johnson & Johnson/ Ethicon).

After sacrifice, the right hemimandible was removed and fixed in 10% formalin for 48 hours, demineralized in 18% EDTA for a period of about 40 days, processed, and embedded in paraffin. Semiserial sections 6 μm thick were obtained in a transversal direction. The sections were stained with either hematoxylin-eosin (H&E) or Masson trichrome for histologic and histometric analysis.

### Histologic and Histometric Analysis

Histologic analysis was performed in all semiserial stained sections of each specimen in both groups. The histologic sections were blindly analyzed by a single calibrated observer with an Olympus CH2 binocular microscope (Olympus Optical, Tokyo, Japan) for the following: presence of granulation tissue, presence of blood clot, osteogenic activity, occurrence of resorption, newly formed bone areas, reorganization of the periosteum, and fulfillment of medullar spaces of bone graft. For the histometric analysis, the 2 most central histologic sections of each specimen block and experimental period were selected (excluding those in center with a drilled hole), photographed with a digital camera (Olympus DP 10; Olympus Optical, Tokyo, Japan) connected to a light microscope (Olympus BX 50 F4; Olympus Optical), and then transferred to a computer. Measurements were performed by image analysis software SigmaScan Pro Version 2.0 (Jandel, San Rafael, CA). A kappa test was used to check the validity and reliability of the results. To standardize histometric analysis, the total area of interface and the newly formed bone area were obtained in pixels. The data were converted to percentages (ratio of newly formed bone/interface area) and submitted to statistical analysis. The significance of differences between the groups about the percentage of newly formed bone of the interface area were determined by analysis of variance followed by a post hoc Tukey test when the analysis of variance suggested a significant difference between groups ($P < .05$).

## RESULTS

### Descriptive Histologic Analysis

*7 Days*. In the saline group, granulation tissue surrounding the external bony graft surface and invading the bed-graft interface was observed. The tissue

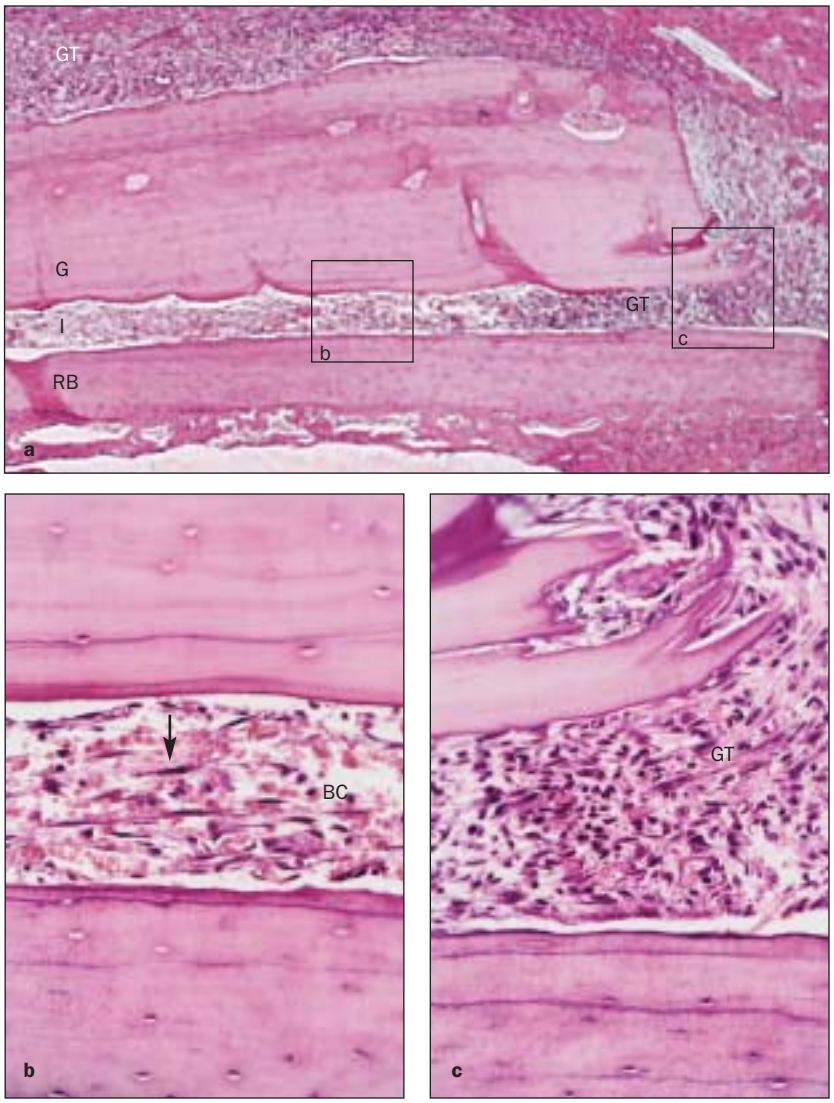


**Fig 1** Saline group, 7 days. *(a)* Granulation tissue (GT) surrounding the external bony graft (G) surface and invading the bed-graft interface (I). RB = receptor bed (H&E, original magnification ×38). *(b)* Detail of the graft periphery and the GT characterized by a significant amount of blood vessels and numerous fibroblast-like cells (H&E, original magnification ×275). *(c)* Detail of the central area of 1a filled with blood clot (BC) and invaded by fibroblast-like cells (*black arrow*; H&E, original magnification ×275).

was characterized by a significant amount of blood vessels and numerous fibroblast-like cells. The most central area of the interface presented filled with blood clot and, at times, small bone splinters. In some specimens, a discrete osteogenic activity was observed in the bony surface of the bed recipient facing the graft (Fig 1).

In the nicotine group, the external bony graft surface was involved with immature, poorly cellularized granulation tissue. Blood clot was observed along most of the bed-graft interface, and, in a number of specimens, it was invaded by fibroblast-like cells originating from the lateral periphery (Fig 2).

*14 Days.* The saline group demonstrated a significant amount of woven bone formation, with numerous osteocytes in the interface. Osteoblasts were observed adjacent to the borders of the newly formed bony tissue, with collagen fibers inserted between the cells. Areas of connective tissue interposition between graft and bed recipient were also observed. The graft was intertwined with a connective tissue rich in fibers and cells and arranged in a parallel fashion, resembling periosteum (Fig 3).

The majority of nicotine-group specimens demonstrated an interface filled with a connective tissue poor in collagen fibers and connective tissue. In some areas, woven bone islets originating from the receptor bed were also seen, sometimes in association with small bone splinters. The connective tissue seen at the graft periphery was poorly organized (Fig 4).

*28 Days.* In the saline group, the graft-bed interface showed intense trabecular bone formation connecting the bed and the lower surface of the graft. However, some areas of connective tissue were interposed between the graft and bed. The majority of intertrabecular spaces was filled by a myeloid tissue in early development (Fig 5).

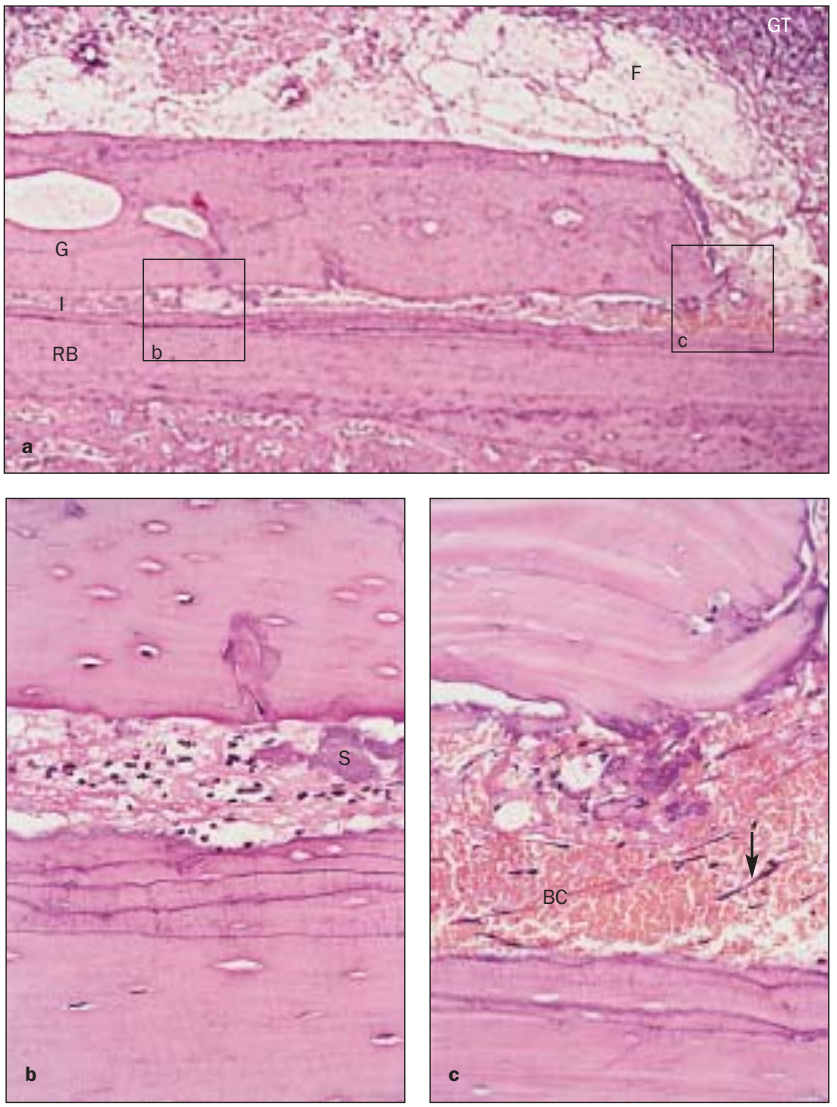


**Fig 2** Nicotine group, 7 days. *(a)* External bony graft (G) surface surrounded by a net of fibrin (F) and immature granulation tissue (H&E, original magnification ×47). *(b)* Detail of the central area of 2a filled by a net of fibrin and blood cells in apoptosis and small bone splinters (S; H&E, original magnification ×275). *(c)* Detail of the graft periphery and the blood clot (BC) and a discrete migration of fibroblast-like cells (*black arrow*; H&E, original magnification ×275).

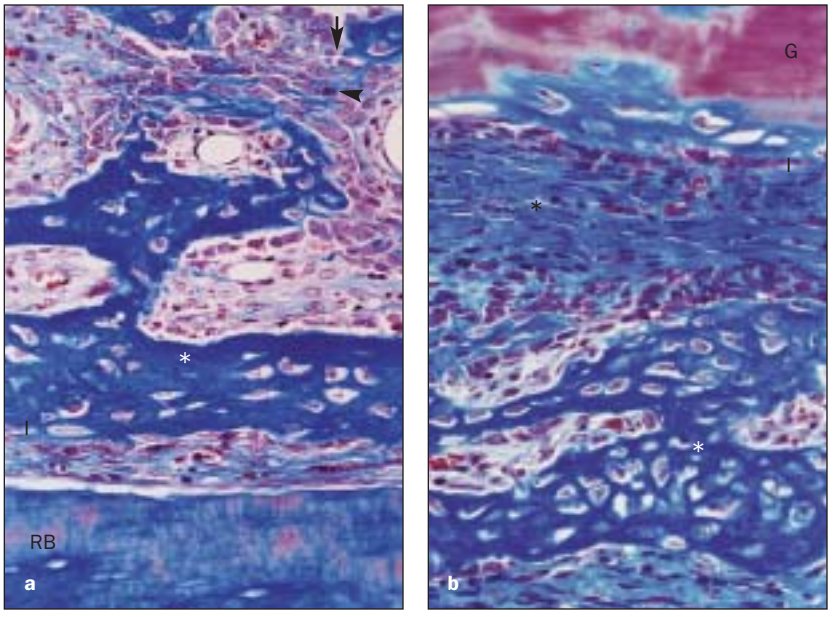


**Fig 3** Saline group, 14 days. *(a)* Detail of the bone neoformation *(white asterisk)* and osteoblasts *(black arrowhead)* adjacent to the newly formed bony tissue and the collagen fibers *(black arrow)*. *(b)* Detail of the bone neoformation *(white asterisk)* and the connective tissue *(black asterisk)* between the graft (G) and the newly formed bony tissue (Masson trichrome, original magnification ×275).

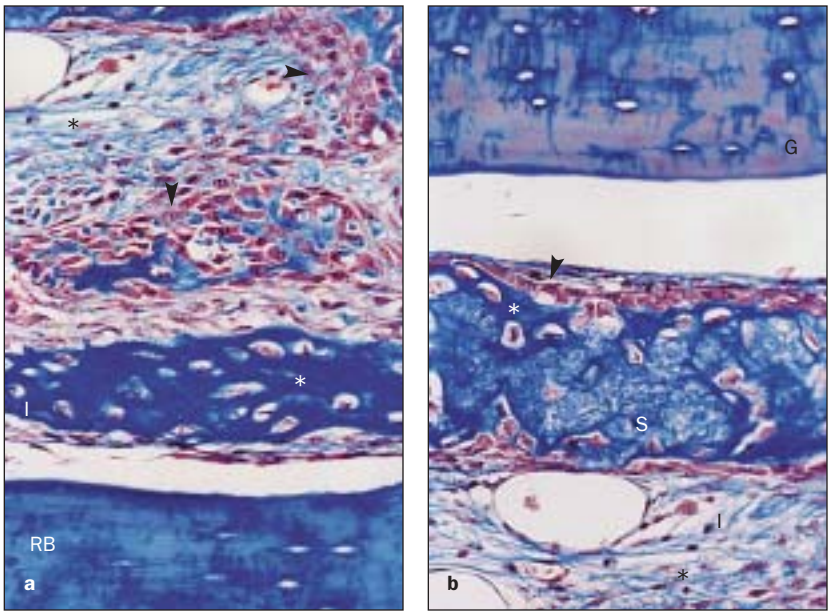


**Fig 4** Nicotine group, 14 days. *(a)* Detail of woven bone islets *(white asterisk)* near the receptor bed (RB), the osteoblasts *(arrowhead),* and the connective tissue (*black asterisk*; Masson trichrome, magnification ×275). *(b)* Detail of the bed-graft interface filled with poor collagen fibers, fibroblast-like cells, connective tissue, and bone neoformation *(white asterisk)* associated with bone splinters (S) and osteoblasts (*arrowhead*; Masson trichrome, original magnification ×275).

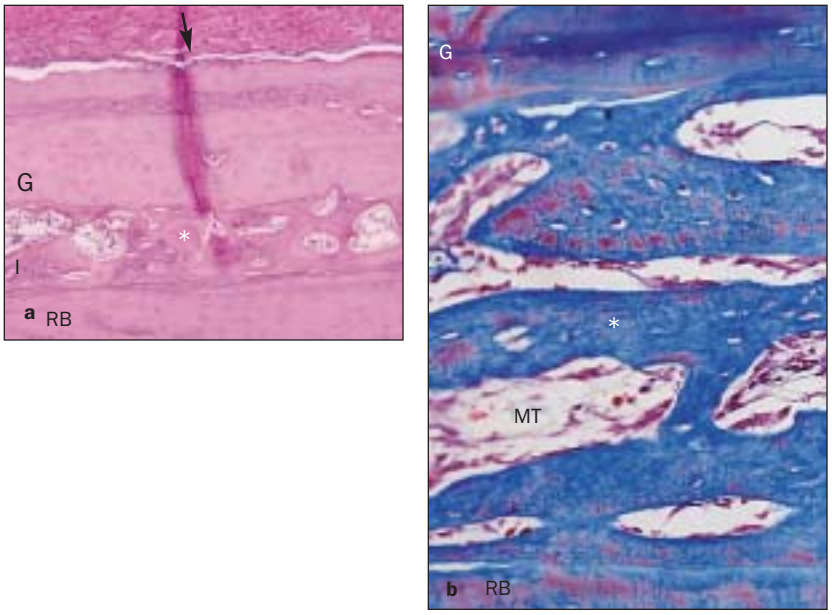


**Fig 5** Saline group, 28 days. *(a)* Intense trabecular bone formation *(white asterisk)* was observed, filling the bed-graft interface and the external bony graft surface, which was surrounded by a connective tissue similar to periosteum (*arrow*; H&E, magnification ×87). *(b)* Detail of bone formation connecting the bed and the lower surface of the graft *(white asterisk)* and the intertrabecular spaces, which were filled by a myeloid tissue in early development (Masson trichrome, original magnification ×275).

Most of the nicotine-group specimens exhibited areas of trabecular bone formation interposed by connective tissue poor in collagen fibers and fibroblast-like cells. Only 2 specimens presented intense trabecular bone formation in the interface, with the majority of intertrabecular spaces filled by connective tissue (Fig 6).

## Statistical Analysis

The kappa test showed validity and reliability of 95% for the calibrated observer. ANOVA showed significant differences between groups at the 5% level. These differences were found between treatment groups ($P < .001$), and between healing periods ($P = .009$), but not between both of them ($P = .8367$).

The differences between treatment groups and healing periods were examined with the Tukey test (Table 1).

Both groups showed bone tissue neoformation at the graft-native bone interface during the period of observation (Table 1). However, comparison of the results between the saline and nicotine groups revealed that bone neoformation was significantly greater in the saline group in comparison to the nicotine group (Table 1).

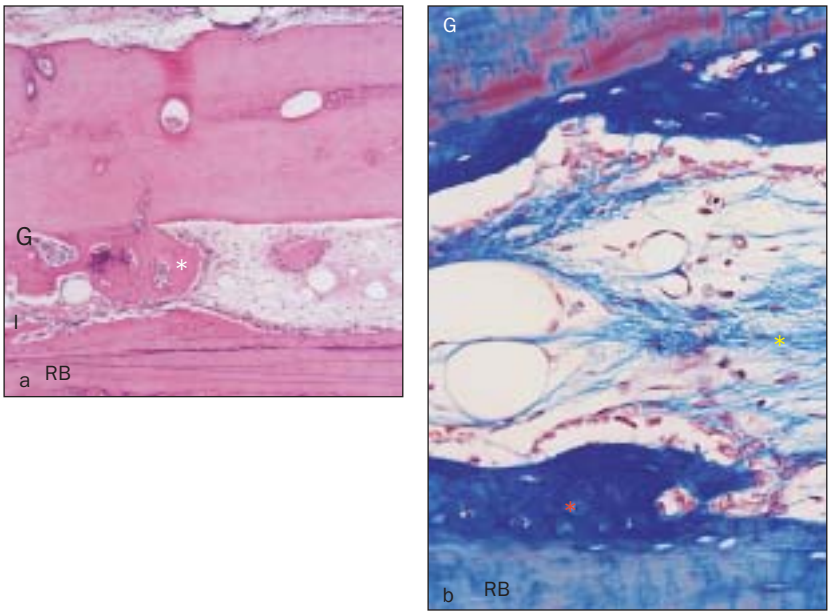


**Fig 6** Nicotine group, 28 days. *(a)* Observe the trabecular bone formation and external bony surface surrounded by connective tissue (H&E, original magnification ×87). *(b)* Detail of the bone neoformation *(red asterisk)* near the graft and recipient bed and the connective tissue *(yellow asterisk).*

**Table 1 Mean ± SD Amount of Newly Formed Bone as a Percentage of the Total Area of Interface**

| | Healing period | | | | | |
|---|---|---|---|---|---|---|
| | 14 days | | | 28 days | | |
| Group | Mean | SD | Sig | Mean | SD | Sig |
| Saline | 51.31 | 8.31 | aB | 73.00 | 4.99 | aA |
| Nicotine | 23.75 | 6.18 | bB | 42.44 | 8.70 | bA |

Means ± SD followed by different letters (lower case letters in columns and capital letters in lines) are significantly different (Tukey test; $P < .05$).

## DISCUSSION

Jardini et al[26] used male rats (250 to 300 g) to analyze the biologic mechanisms and the timing sequence of healing events of autogenous bone block grafts obtained from the calvaria and affixed to the mandible. Based on the same methodology, the aim of this study was to perform qualitative and quantitative analyses of the influence of nicotine on autogenous bone block grafts, emphasizing the timing sequence of the initial (early) healing process and the differences in the repair processes.

The method employed for bone block fixation was the well-established protocol of Jardini et al.[26] In a rat model, a tight suture was used to stabilize the bone block graft, avoiding micromovements and thus allowing bony union. In a previous unpublished pilot study, the use of screws for fixation of the bone block graft was found to be impractical because of fracture of the recipient area due to the thinness of the rat mandible.

The methodology used for autogenous bone block grafting was chosen based on the successful experimental model described by Jardini et al[26] to determine the early healing pattern of autogenous bone block grafts and reproduced by De Marco et al[27] to study revascularization. The animal model selected was found to be convenient in addition to being well-established. At this time, to the authors' knowledge, no studies have examined the healing process of autogenous bone block grafts in rats submitted to systemic administration of nicotine.

The choice of graft type affected graft resorption. Revascularization of cortical graft is slower than that of cancellous bone and occurs through preexisting havesian channels. The remodeling is also slow and results in a blend of necrotic and newly formed bone.[28]

Experimental studies in animals have used nicotine added to drinking water,[15] nicotine patches and nasal spray with nicotine,[29] and mini-osmotic pumps.[30] In the present study, nicotine was administered via subcutaneous injection, which produced serum levels similar to those of human smokers who consume 10 to 20 cigarettes/d.[31] However, comparisons with humans should be made with caution because of differences in the metabolism of nicotine between humans and rats. Even though administration of nicotine may start immediately after surgical procedures,[32,33] it seemed clinically relevant to previously expose an organism to the drug by beginning the administration 4 weeks prior to surgical procedures.[17]

The 7-day results showed that in the saline group, the graft was interspersed with a typical granulation tissue, with a significant amount of fibroblast-like cells and many blood vessels. In the nicotine group,

the graft was surrounded by an immature granulation tissue associated with a net of fibrin. In addition, the bed-graft interface presented a significant amount of blood clot associated with a discrete migration of fibroblast-like cells from the lateral borders. These initial findings are most likely explained by the inhibitory effect of nicotine on the maturation and proliferation capacity of erythrocytes. This effect may be related to the enhanced combination of carbon monoxide with hemoglobin in the blood, also caused by nicotine, which prevents the hemoglobin from carrying adequate oxygen to the various organs and tissues of the body.[34] In addition, blood flow diminishes as a function of the vasoconstrictive properties of nicotine, as well as the stimulation of sympathetic ganglia and the adrenal medulla, which result in an increase of epinephrine and norepinephrine discharge.[35] Nicotine can not only interfere with the early revascularization, which is important in providing nutrition to the surviving cells, but also delay the establishment of vessels within the bone graft, reducing the area of revascularization.[12]

At 14 days, the histometric analyses indicated that the newly formed bone area at the native bone-graft interface was significantly greater in bone grafts for the saline group compared with the nicotine group (51.31% ± 8.31% versus 23.75% ± 6.18%, respectively). This pattern could be also observed in the histologic analyses, which depicted intense bone formation in the saline group in contrast to discrete osteogenic activity in the nicotine group. The delay in the timing sequence of healing events can be related to the lack of osteoblastic activity, which led to decreased bone formation.[12–14,17,36,37] In addition, the presence of connective tissue poor in collagen fibers and fibroblast-like cells with nicotine exposure has also been found in in vitro studies, which also demonstrated decreased proliferation[38–40] and cellular migration,[38,39] together with the inhibition of fibronectin and type I collagen production.[40] The presence of nicotine in the fibroblast's intracellular compartment is likely to disrupt normal cellular metabolic processes and consequently impair the reparative and regenerative potential of tissues.[10,39]

After 28 days, most of the specimens of the saline group showed signs of incorporation, as revealed by bone bridges connecting the receptor bed to the lower surface of the graft. The process of incorporation is primarily a result of peripheral osteoclastic resorption and vascular infiltration of Volkmann and haversian channels.[41] In contrast, in the nicotine group, the interface was generally partially filled with connective tissue, with some areas of newly formed bone bridging the recipient bed to the graft. The histometric results confirmed these findings, showing significantly less bone formation in the nicotine group compared with the saline group (42.44± 8.70 versus 73.00± 4.99).

In accordance with the literature, the results of the present study showed a decrease in new bone apposition and mineralization of the bone matrix[37] and a decrease in bone cell growth and differentiation.[36,42]

Some investigators[32,43] have proposed the use of nicotine patches because nicotine does not prevent bone healing. However, this procedure should be considered with caution, because several studies show that nicotine is able to influence bone healing.[12,13,15,17,35] Recently, studies from the authors' laboratory showed that nicotine (not smoking) increased periodontal bone loss in rats, regardless of plaque infection.[19] All of these findings underscore the importance of abstaining from nicotine-containing products prior to and following osseous procedures.

The limits of extrapolating the results of this study to clinical situations should be considered. It could be speculated that smokers and users of nicotine patches should need a longer postoperative healing period for the complete incorporation of bone graft. However, further investigation is needed to clarify the long-term consequences of nicotine on bone graft healing, the effect of different doses of the drug, and the behavior of the drug in different experimental models.

## CONCLUSION

In conclusion, nicotine did jeopardize the healing process of autogenous bone block grafts in rats but did not prevent bone healing.

## ACKNOWLEDGMENTS

The authors wish to express special thanks to Dr Rumio Taga and Dr Tania Mary Cestari for their expert help with histologic analysis and to Dr Sílvia Helena Venturoli Perri for the statistical analysis of the results.

## REFERENCES

1. Bergstrom J, Preber H. Tobacco use as a risk factor. J Periodontol 1994;65:545–550.
2. Calsina G, Ramon JM, Echeverria JJ. Effects of smoking on periodontal tissues. J Clin Periodontol 2002;29:771–776.
3. Grossi SG, Zambon J, Machtei EE, et al. Effects of smoking and smoking cessation on healing after mechanical periodontal therapy. J Am Dent Assoc 1997;128:599–607.
4. Renvert S, Dahlén G, Wikström M. The clinical and microbiological effects of non-surgical periodontal therapy in smokers and non-smokers. J Clin Periodontol 1998;25:153–157.

5. Labriola A, Needleman I, Moles DR. Systematic review of the effect of smoking on nonsurgical periodontal therapy. Periodontol 2000 2005;37:124–137.
6. Kinane DF, Radvar M. The effect of smoking on mechanical and antimicrobial periodontal therapy. J Periodontol 1997;68:467–472.
7. Scabbia A, Cho KS, Sigurdsson TJ, Kim CK, Trombelli L. Cigarette smoking negatively affects healing response following flap debridement surgery. J Periodontol 2001;72:43–49.
8. Trombelli L, Cho KS, Kim CK, Scapoli C, Scabbia A. Impaired healing response of periodontal furcation defects following flap debridement surgery in smokers. A controlled clinical trial. J Clin Periodontol 2003;30:81–87.
9. Martins AG, Andia DC, Sallum AW, Sallum EA, Casati MZ, Nociti FH Jr. Smoking may affect root coverage outcome: A prospective clinical study in humans. J Periodontol 2004;75:586–591.
10. Hanes PJ, Schuster GS, Lubas S. Binding, uptake, and release of nicotine by human gingival fibroblasts. J Periodontol 1991;62:147–152.
11. Tonetti MS. Cigarette smoking and periodontal diseases: Etiology and management of disease. Ann Periodontol 1998;3:88–101.
12. Daftari TK, Whitesides TE Jr, Heller JG, Goodrich AC, McCarey BE, Hutton WC. Nicotine on the revascularization of bone graft. An experimental study in rabbits. Spine 1994;15;19:904–911.
13. Riebel GD, Boden SD, Whitesides TE, Hutton WC. The effect of nicotine on incorporation of cancellous bone graft in an animal model. Spine 1995;20:2198–2202.
14. Silcox DH III, Daftari T, Boden SD, Schimandle JH, Hutton WC, Whitesides TE Jr. The effect of nicotine on spinal fusion. Spine 1995;20:1549–1553.
15. Hollinger JO, Schmitt JM, Hwang K, Soleymani P, Buck D. Impact of nicotine on bone healing. J Biomed Mater Res 1999;45:294–301.
16. Theiss SM, Boden SD, Hair G, Titus L, Morone MA, Ugbo J. The effect of nicotine on gene expression during spine fusion. Spine 2000;25:2588–2594.
17. Pinto JR, Bosco AF, Okamoto T, Guerra JB, Piza IG. Effects of nicotine on the healing of extraction sockets in rats. A histological study. Braz Dent J 2002;13:3–9.
18. Saldanha JB, Pimentel SP, Casati MZ, et al. Guided bone regeneration may be negatively influenced by nicotine administration: A histologic study in dogs. J Periodontol 2004;75:565–571.
19. Bosco AF, Bonfante S, de Almeida JM, Luize DS, Nagata MJ, Garcia VG. A histologic and histometric assessment of the influence of nicotine on alveolar bone loss in rats. J Periodontol 2007;78:527–532.
20. Olson JW, Dent CD, Morris HF, Ochi S. Long-term assessment (5 to 71 months) of endosseous dental implants placed in the augmented maxillary sinus. Ann Periodontol 2000;5:152–156.
21. Bain CA, Moy PK. The association between the failure of dental implants and cigarette smoking. Int J Oral Maxillofac Implants 1993;8:609–615.
22. Widmark G, Andersson B, Carlsson GE, Lindvall AM, Ivanoff CJ. Rehabilitation of patients with severely resorbed maxillae by means of implants with or without bone grafts: A 3- to 5-year follow-up clinical report. Int J Oral Maxillofac Implants 2001;16:73–79.
23. Kan JY, Rungcharassaeng K, Lozada JL, Goodacre CJ. Effects of smoking on implant success in grafted maxillary sinuses. J Prosthet Dent 1999;82:307–311.
24. Levin L, Schwartz-Arad D. The effect of cigarette smoking on dental implants and related surgery. Implant Dent 2005;14:357–361.
25. Okamoto M, Kita T, Okuda H, Tanaka T, Nakashima T. Effects of aging on acute toxicity of nicotine in rats. Pharmacol Toxicol 1994;75:1–6.
26. Jardini MA, De Marco AC, Lima LA. Early healing pattern of autogenous bone grafts with and without e-PTFE membranes: A histomorphometric study in rats. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;100:666–673.
27. De Marco AC, Jardini MA, Lima LP. Revascularization of autogenous block grafts with or without an e-PTFE membrane. Int J Oral Maxillofac Implants 2005;20:867–874.
28. Burchardt H. Biology of bone transplantation. Orthop Clin North Am 1987;18:187–196.
29. Sipe RV III, Buck DC, Hollinger JO. Nicotine administration in rabbits using Habitrol nicotine patches and nicotine nasal spray. Clin Exp Pharmacol Physiol 2000;27:480–482.
30. Iwaniec UT, Fung YK, Akhter MP, et al. Effects of nicotine on bone mass, turnover, and strength in adult female rats. Calcif Tissue Int 2001;68:358–364.
31. Gonzalez YM, De Nardin A, Grossi SG, Machtei EE, Genco RJ, De Nardin E. Serum cotinine levels, smoking, and periodontal attachment loss. J Dent Res 1996;75:796–802.
32. Stefani CM, Nogueira F, Sallum EA, de TS, Sallum AW, Nociti FH Jr. Influence of nicotine administration on different implant surfaces: A histometric study in rabbits. J Periodontol 2002;73:206–212.
33. Cesar-Neto JB, Duarte PM, Sallum EA, Barbieri D, Moreno H Jr, Nociti FH Jr. A comparative study on the effect of nicotine administration and cigarette smoke inhalation on bone healing around titanium implants. J Periodontol 2003;74:1454–1459.
34. Mosely LH, Finseth F, Goody M. Nicotine and its effect on wound healing. Plast Reconstr Surg 1978;61:570–575.
35. Cryer PE, Haymond MW, Santiago JV, Shah SD. Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. N Engl J Med 1976;295:573–577.
36. Fang MA, Frost PJ, Iida-Klein A, Hahn TJ. Effects of nicotine on cellular function in UMR 106-01 osteoblast-like cells. Bone 1991;12:283–286.
37. Fung YK, Iwaniec U, Cullen DM, Akhter MP, Haven MC, Timmins P. Long-term effects of nicotine on bone and calciotropic hormones in adult female rats. Pharmacol Toxicol 1999;85:181–187.
38. Giannopoulou C, Geinoz A, Cimasoni G. Effects of nicotine on periodontal ligament fibroblasts in vitro. J Clin Periodontol 1999;26:49–55.
39. James JA, Sayers NM, Drucker DB, Hull OS. Effects of tobacco products on the attachment and growth of periodontal ligament fibroblasts. J Periodontol 1999;70:518–525.
40. Tipton DA, Dabbous MK. Effects of nicotine on proliferation and extracellular matrix production of human gingival fibroblasts in vitro. J Periodontol 1995;66:1056–1064.
41. Enneking WF, Burchardt H, Puhl JJ, Piotrowski G. Physical and biological aspects of repair in dog cortical-bone transplants. J Bone Joint Surg Am 1975;57:237–252.
42. Yuhara S, Kasagi S, Inoue A, Otsuka E, Hirose S, Hagiwara H. Effects of nicotine on cultured cells suggest that it can influence the formation and resorption of bone. Eur J Pharmacol 1999;383:387–393.
43. Nociti Júnior FH, Cesar Neto JB, Carvalho MD, Sallum EA, Sallum AW. Intermittent cigarette smoke inhalation may affect bone volume around titanium implants in rats. J Periodontol 2002;73:982–987.