

Effects of Nicotine on the Healing of Extraction Sockets in Rats. A Histological Study

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The aim of the present study was to evaluate, histologically, the influence of nicotine on the socket healing after tooth extraction in rats. Eighty animals were divided into 4 groups of 20 rats each (2 control and 2 test groups). In the first and second test groups, the animals received one dose of nicotine hemisulfate solution once or twice daily, respectively. In the control groups, the animals received sterile saline once or twice daily. All solutions were injected subcutaneously on the dorsum of the animals for 4 weeks. The results showed that nicotine delayed alveolar healing, especially in terms of organization of connective tissue and osteoneogenesis. Angiogenesis was considerably impaired in the ossification area and in the gingival tissues as well. We concluded that the impairment of the healing of extraction sockets was found to be directly related to the drug dosage.

Key Words: nicotine, alveolar healing, angioneogenesis, osteoneogenesis.

INTRODUCTION

Nicotine, the major alkaloid of tobacco leaves (*Nicotiana tabacum*), is one of the drugs most extensively consumed in the world. It has well-known harmful effects on phenomena related to tissue healing, such as inhibition of angiogenesis (1), re-epithelialization (2,3) and osteogenesis (4,5) as well as cellular healing, such as inhibition of fibroblast proliferation and adhesion (6,7) and of collagen synthesis (8,9).

Alveolar healing has been studied extensively and has thus become a well-known process. However, little information is available in dental literature concerning the influence of nicotine on alveolar healing. Therefore, the aim of the present study was to investigate histologically the systemic action of nicotine on the healing of tooth extraction wounds in rats.

MATERIAL AND METHODS

Eighty male Wistar rats (*Rattus norvegicus*

albinus), aged 120 days and weighing 280 to 320 g, were divided into four groups of 20 rats each, two of them were considered experimental (test 1 and test 2) and two were used as controls (control 1 and control 2). The animals were kept in their respective cages at 24-27°C with automatic light control (5:00 a.m. to 7:00 p.m.) and free access to food and water.

Nicotine hemisulphate (Sigma Chemical Co., St. Louis, MO, USA) was diluted in saline at the concentration of 5 mg/ml for the experimental groups. Each rat received a volume of the diluted solution calculated as 3 g/kg weight according to the procedure proposed by Okamoto et al. (10).

The animals in test 1 group received only one dose of the drug administered once a day, while the animals in test 2 group received two doses at 12-h intervals. The control groups (1 and 2) received saline under the same conditions as the test groups. All animals received either nicotine or saline for a period of 4 weeks before tooth extraction. No solutions were administered on the day of tooth extraction.

The animals were anesthetized intraperitoneally with sodium pentobarbital (46 mg/kg body weight), prepared in a solution of 20 mg/ml of the anesthetic per 90% saline plus 5% absolute alcohol. The right maxillary incisor was extracted using a plier for residual roots that was adapted by Okamoto and Russo (11) by grinding the external surfaces of the plier tips and by increasing the concavity of their internal surfaces in order to hold the incisor of the rat. The margins of the wounds were sutured using 4.0 silk sutures. After surgery, a single dose of 30,000 IU penicillin-G benzathin was administered intramuscularly.

The animals continued to receive their respective solution until the time of sacrifice (3, 7, 15 and 28 days after tooth extraction). The animals were sacrificed by excess sulphuric ether inhalation. The right maxilla was separated from the left by a median sagittal incision. Another incision was made tangential to the distal surface of the molars to obtain a block containing the right incisor alveolus. The blocks were fixed in neutral 10% formalin and decalcified in equal parts of 20% sodium citrate and 50% formic acid. After routine laboratory processing, the blocks were embedded in paraffin for bucco-lingual alveolar sections. Semi-serial 6- μ m thick sections were obtained and stained with hematoxylin and eosin for examination with a light microscope.

RESULTS

The two control groups, although analyzed individually, will be described as a single group (control) because no differences were observed between them.

3 days

Control groups: The dental alveolus was almost fully filled by a blood clot containing numerous macrophages. The presence of neofomed capillaries and fibroblasts was clearly visible in the remnant periodontal ligament (Figure 1). The epithelium of the gingival mucosa, with discontinuity, showed moderate proliferation and the adjacent connective tissue exhibited neofomed capillaries, some fibroblasts and some macrophages.

Test 1 group: Almost the entire extent of the dental alveolus was filled with a blood clot containing numerous macrophages. A moderate number of fibro-

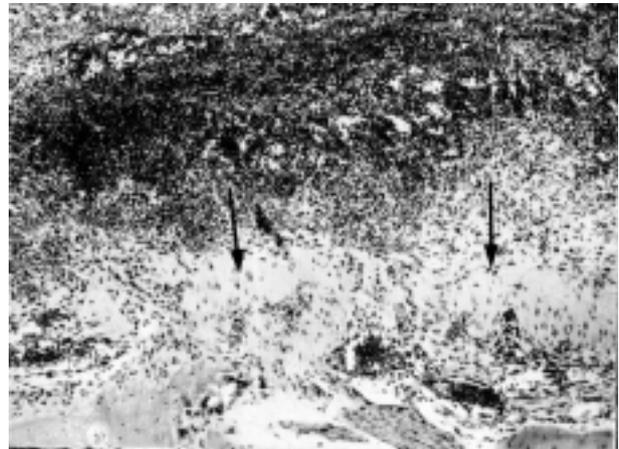


Figure 1. Control group. 3 days. Middle third of the socket presents a well vascularized periodontal ligament, with many fibroblasts (arrows). H.E. Original magnification, 63x.

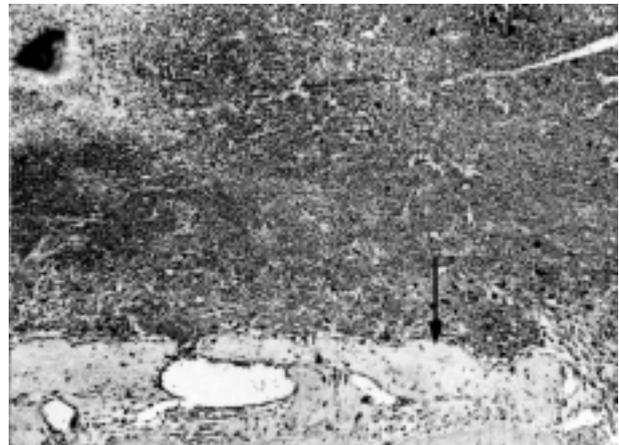


Figure 2. Test 1 group. 3 days. Middle third of the socket presents the periodontal ligament (arrow) with a moderate number of fibroblasts and blood vessels. H.E. Original magnification, 63x.



Figure 3. Test 2 group. 3 days. Middle third of the socket presents the periodontal ligament (arrow) with a discrete number of fibroblasts and blood vessels. H.E. Original magnification, 63x.

blasts and blood vessels were present close to the remnant periodontal ligament (Figure 2). The epithelium of the gingival mucosa, with discontinuity, showed discrete cell proliferation. No neoformed capillaries were observed.

Test 2 group: The dental alveolus was almost completely filled by a blood clot, which contained a moderate number of macrophages. In the remaining periodontal ligament, the amount of fibroblasts and neoformed vessels was still quite small (Figure 3). The epithelium of the gingival mucosa, with discontinuity, showed discrete proliferation. The underlying connective tissue showed no evidence of angiogenesis.

7 days

Control groups: The dental alveolus was partially filled by well-vascularized neoformed connective tissue rich in fibroblasts, especially close to the alveolar bone walls (Figure 4). Small neoformed bone trabeculae were present, with numerous osteoblasts on their margins. The epithelium of the gingival mucosa almost covered the dental alveolus and the underlying connective tissue exhibited a pronounced neovascular network.

Test 1 group: Neoformed connective tissue showed a smaller number of fibroblasts and blood vessels compared to the control group (Figure 5). There were small neoformed bone spicules near the bone walls. The epithelium of the gingival mucosa exhibited a discrete discontinuity, and vascular formation in the underlying connective tissue was less developed than

in the control group.

Test 2 group: There was still a large amount of blood clot containing numerous macrophages throughout the dental alveolus. Neoformed connective tissue was observed, with similar characteristics to those observed in test 1 group. Rare neoformed bone spicules were observed near the bone walls, but the general picture was still characterized by an absence of osteoneogenesis (Figure 6). The epithelium of the gingival mucosa showed discrete discontinuity and the connective tissue was similar to the test 1 group, however with fewer neoformed blood vessels.

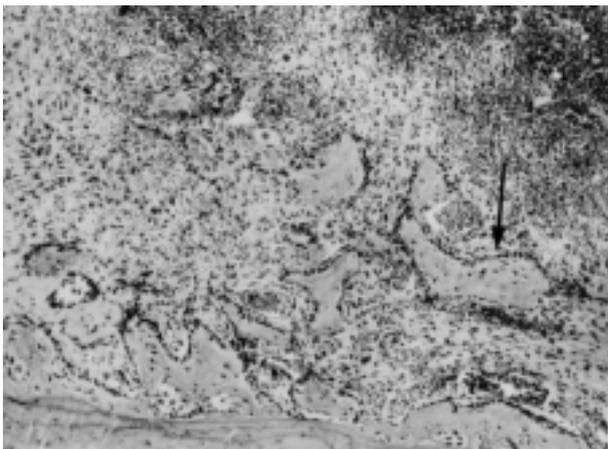


Figure 4. Control group. 7 days. Middle third of the socket presents small neoformed bone trabeculae (arrow). H.E. Original magnification, 63x.

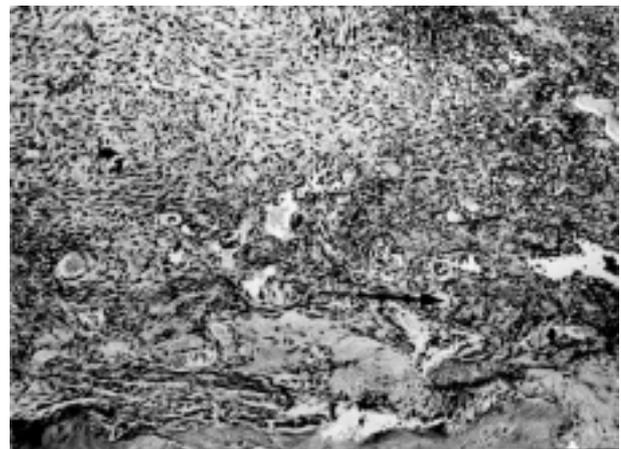


Figure 5. Test 1 group. 7 days. Middle third of the socket presents small neoformed bone spicules (arrow). H.E. Original magnification, 63x.

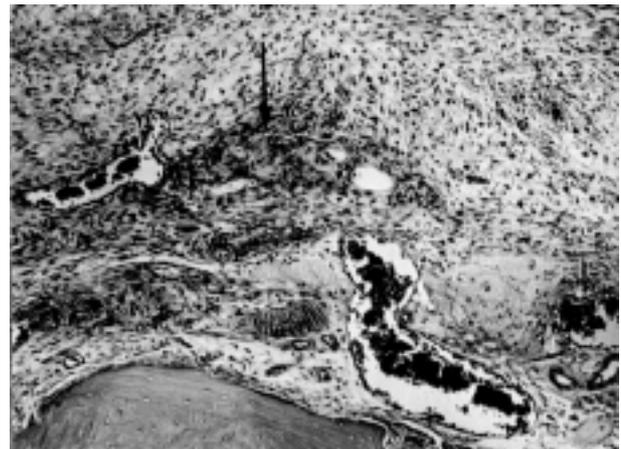


Figure 6. Test 2 group. 7 days. Middle third of the socket presents neoformed bone spicules (arrow). H.E. Original magnification, 63x.

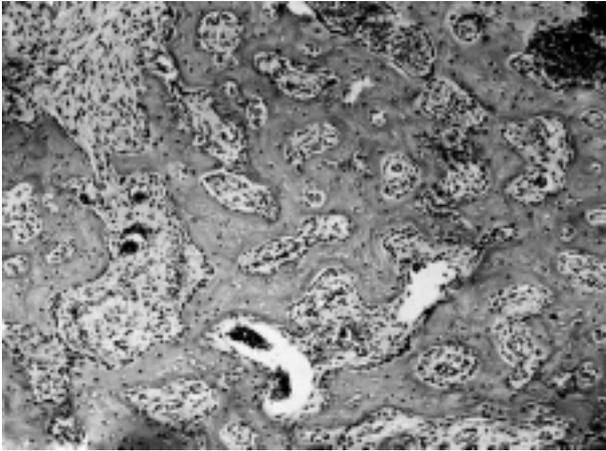


Figure 7. Control group. 15 days. Middle third of the socket presents regular and thin bone trabeculae. H.E. Original magnification, 63x.

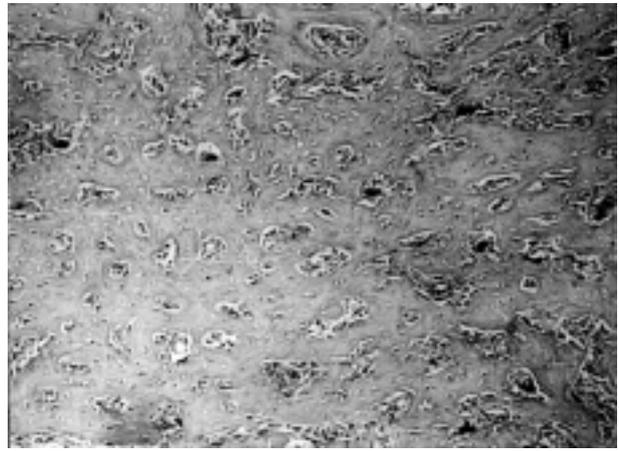


Figure 10. Control group. 28 days. Middle third of the socket presents thick and well-defined bone trabeculae. H.E. Original magnification, 63x.

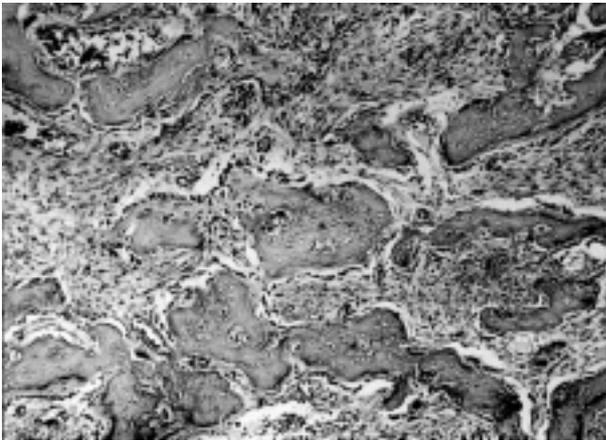


Figure 8. Test 1 group. 15 days. Middle third of the socket presents thin and isolated bone trabeculae. H.E. Original magnification, 63x.

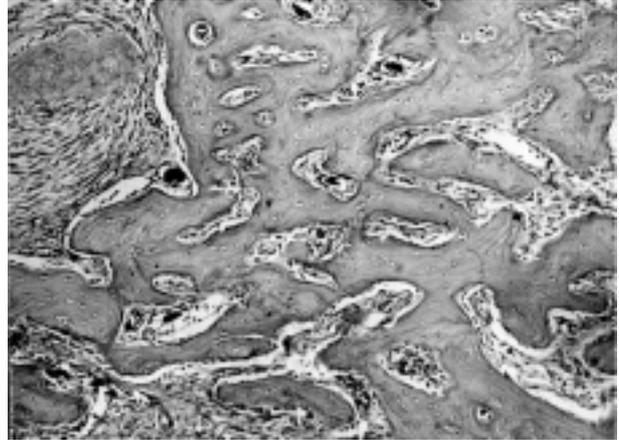


Figure 11. Test 1 group. 28 days. Middle third of the socket presents thick and thin bone trabeculae. H.E. Original magnification, 63x.

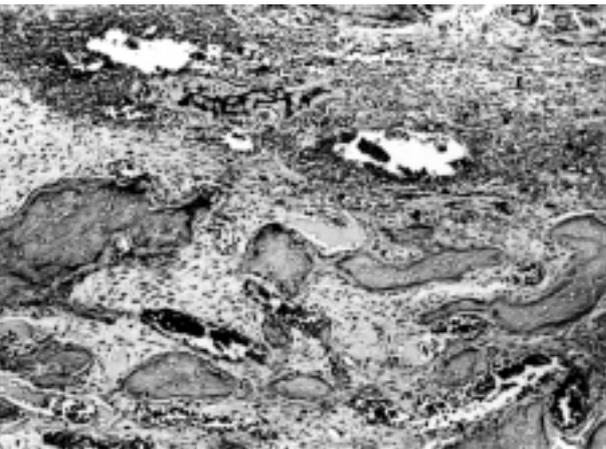


Figure 9. Test 2 group. 15 days. Middle third of the socket presents thin bone trabeculae and a large amount of connective tissue without bone differentiation. H.E. Original magnification, 63x.

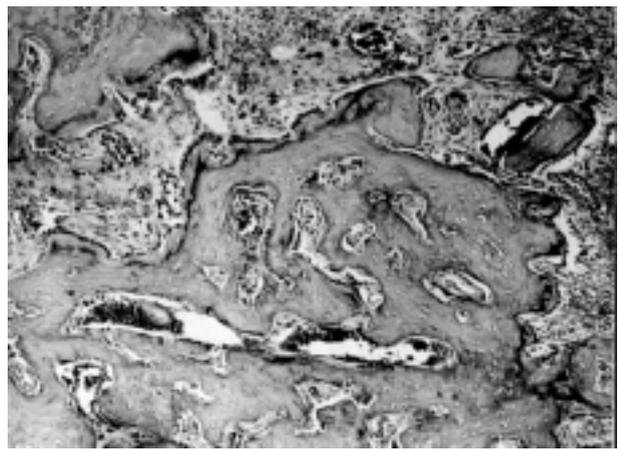


Figure 12. Test 2 group. 28 days. Middle third of the socket presents fine bone trabeculae. H.E. Original magnification, 63x.

15 days

Control groups: Except for several sites presenting some remaining blood clot, the dental alveolus was mostly filled with regular and thin bone trabeculae (Figure 7). The epithelium of the gingival mucosa fully covered the dental alveolus and the underlying connective tissue was well developed.

Test 1 group: Thin and isolated bone trabeculae were observed in the midst of large amounts of connective tissue with areas of clot (Figure 8). The epithelium of the gingival mucosa covered the dental alveolus and the underlying connective tissue showed characteristics similar to those observed in the 7 day control groups.

Test 2 group: There was a predominance of poorly vascularized connective tissue with a small number of scattered fibroblasts and few and small neoformed bone spicules (Figure 9). As also observed in the test 1 group, there was a larger amount of remaining clot areas than in the controls. The epithelium of the gingival mucosa completely covered the alveolus but the underlying connective tissue was still not well organized, being less dense and vascularized than in the test 1 group.

28 days

Control groups: The dental alveolus was completely filled with thick and well-defined trabecular bone (Figure 10). The alveolar bone crista was remodeled. The gingival mucosa was fully regenerated, as already observed in the control group at 15 days.

Test 1 group: Osteoneogenesis was still discrete, with the presence of fine trabecular bone and the persistence of large amounts of connective tissue without bone differentiation (Figure 11). Ossification was more intense in some areas, exhibiting well-organized, at times fine and at times thick trabecular bone.

Test 2 group: The extent of ossification was clearly less than in the test 1 group, with the presence of fine and isolated bone trabeculae in the midst of a large amount of connective tissue without bone differentiation (Figure 12). Few sites exhibited well developed trabecular bone. At this time, both the test 1 and test 2 groups showed conditions of epithelium and gingival connective tissue similar to those observed in the control groups, although a smaller amount of blood vessels was clearly observed.

DISCUSSION

Oral mucosa and epithelial lining regeneration, which had been already satisfactorily completed 7 days post-operative in the control animals, was observed in the experimental animals only after 15 days, in agreement with Mosely et al. (3), who reported a significant delay in the healing process of wounds in the ear of rabbits injected with nicotine compared to controls. The delay may be associated with inhibition of epithelialization. Nicotine has been identified as an agent that releases catecholamine, which in turn acts as a cofactor in the formation of calones, which are hormones that inhibit epithelialization (2,3).

A relevant action of nicotine is vasoconstriction, which may predispose to thrombotic microvascular occlusion and consequent tissue ischemia (12). In chronic smokers, nicotine causes a reduced production of prostacyclin, which may have a vasoconstrictor effect (13,14). It also stimulates the carotid and aortic chemoreceptor bodies, causing catecholamine release from the adrenergic and adrenal medulla nerve endings, which induces an increase in blood pressure with a consequent oxygen demand, thus impairing the healing process (12). Clinical investigations have pointed out that gingival bleeding, considered to be a factor indicating inflammatory periodontal disease, is decreased in chronic smokers (15,16).

In the present study, the caliber of the blood vessels in the healing area was not evaluated and therefore it is not possible to discuss the influence of a probable reduction of blood flow due to vasoconstriction. However, regarding angiogenesis, the experimental groups had a clear delay in neovascular organization, supporting the findings reported by other investigators who have studied the harmful effects of nicotine in other situations such as revascularization in bone transplants (1), in skin grafts (12), in wound repair (17), and in bone healing (18,19).

In relation to osteoneogenesis, several reports have demonstrated that nicotine administered in toxic doses may delay the ossification of vertebrae and limb bones during fetal development (4,5). In 1991, Fang et al. (6) concluded that moderate doses of nicotine depress cell proliferation of the "osteoblast type", supporting the evidence that this drug has a direct effect on bone cells. In particular, it has been observed that both nicotine and tobacco extract stimulate glycolysis

and inhibit bone synthesis and mitochondrial activity in embryonic cultures of chick tibia (8). Nicotine inhibits the hydroxylation of proline, an indicator of collagen synthesis, in "osteoblast type" cells of the cranial bone of chicks (9). The obvious delay in bone formation in the experimental animals in the present study represents a clear manifestation of the phenomena described by the previously mentioned investigators showing a harmful action of nicotine on osteoblast activity and on osseous collagen synthesis as well. Recently, Scabbia et al. (20) reported that cigarette smoking may negatively influence periodontal healing following flap debridement surgery compared to non-smokers, both in terms of probing depth and clinical attachment level.

At 7 days, while in the control groups the alveolus was almost fully filled by neoformed and well-vascularized connective tissue with the formation of trabecular bone, in the experimental groups the alveolus was only partially filled with neoformed connective tissue, with a smaller number of fibroblasts and blood vessels, and a considerable amount of remaining blood clot. In the test groups, not all specimens exhibited bone formation and when bone was present, it was restricted to some small neoformed spicules close to the bone wall.

At 15 days, while the alveoli of the control groups were filled with trabecular bone in a regular pattern, except for some sites, the alveoli of the experimental groups showed thin and isolated bone trabeculae, with large amounts of poorly vascularized undifferentiated connective tissue, with a reduced number of fibroblasts. In the test 2 group, bone spicules were not even present. At 28 days, the alveoli of the control groups were completely filled with thick trabecular bone, while in the test 1 group the ossification was quite discrete and showed thin trabecular bone with persistence of connective tissue and test 2 group exhibited an even smaller amount of neoformed bone tissue.

We conclude that nicotine acts systemically on the healing of extraction sockets, with an unfavorable action clearly manifested by the delay in bone neoformation and organization and in the regeneration of the gingival mucosa, especially in its connective components. Angiogenesis is clearly impaired in the ossification area and in the gingival chorion as well. The intensity of the harmful effects of nicotine on alveolar healing events is directly related to the dose of the drug.

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RESUMO

Pinto JR, Bosco AF, Okamoto T, Guerra JB, Piza IG. Efeitos da nicotina no processo de reparo de alvéolos dentais de ratos. Estudo histológico. *Braz Dent J* 2002;13(1):3-9.

O objetivo do presente estudo foi avaliar, histologicamente, a influência da nicotina no processo de reparo de alvéolos dentais de ratos. Oitenta animais foram utilizados neste estudo, os quais foram divididos em 4 grupos: 2 controles e 2 tratados. Nos dois grupos tratados, os animais receberam, respectivamente, uma e duas doses diárias de solução de hemissulfato de nicotina. Nos dois grupos controles, os animais receberam solução salina estéril de acordo com o protocolo estabelecido para o grupo tratado. Todas as soluções foram aplicadas subcutaneamente durante todo o período experimental até o dia do sacrifício. Os resultados mostraram que a nicotina atrasa o processo de reparo alveolar, especialmente na organização do tecido conjuntivo e osteogênese. A angiogênese foi consideravelmente prejudicada na área de ossificação assim como no tecido gengival. Dentro dos limites deste estudo, pôde-se concluir que o atraso no processo de reparo do alvéolo foi diretamente relacionado à dosagem da droga injetada.

Unitermos: nicotina, reparo alveolar, angiogênese, osteogênese.

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