Influence of Submandibulectomy on Alveolar Bone Loss in Rats

María I. Vacas,* Mariano Amer,† Ana P. Chiarenza,* María A. Luchelli,* Patricia M. Mandalunis,‡ and Juan C. Elverdin§

Background: The incidence of dry mouth and its public health impact are increasing as the result of a progressively larger, medicated older population and because chronic diseases, like periodontitis, are prevalent pathologies among elderly patients. Periodontitis and continuous remodeling and rebuilding alveolar processes greatly affect the margin of the alveolar bone, and there is evidence indicating the role of submandibular glands in the regulation of immune/inflammatory reactions. The purpose of this study was to assess the effect of submandibular–sublingual complex ablation (Sx) on alveolar bone loss in rats submitted or not to ligature-induced experimental periodontal disease (EP).

Methods: Wistar male rats were submitted to Sx or sham operations (day 0). Two weeks later, unilateral EP was induced on the right mandibular first molars for 7 days with the contralateral side serving as control. Bone loss at the level of the dental pieces was estimated by bone histomorphometry on mesio-distally oriented sections of the molars and by the determination on lingual and vestibular mandibular surfaces of the distances from the cemento-enamel junction to the alveolar crest.

Results: Sx and EP significantly increased lingual and vestibular alveolar bone loss. Molars with EP exhibited greater lingual loss in Sx animals compared to those with the sham operation. EP induced similar interradicular bone loss in sham and Sx rats.

Conclusion: Sx has a deleterious effect on the periodontal tissues, particularly marginal alveolar bone, indicating the importance of the submandibular/sublingual glands in maintaining healthy periodontal conditions. *J Periodontol 2008;79: 1075-1080.*

KEY WORDS

Bone and bones; periodontitis; saliva; salivary glands.

* Department of Biochemistry, Faculty of Dentistry, University of Buenos Aires, Buenos Aires, Argentina.
† Department of Anatomy, Faculty of Dentistry, University of Buenos Aires.
‡ Department of Histology, Faculty of Dentistry, University of Buenos Aires.
§ Department of Physiology, Faculty of Dentistry, University of Buenos Aires.

Saliva is an important protective factor for dentition and soft tissue because of its lubricating, cleansing, antibacterial, buffering, and remineralizing actions.[1] The physiologic importance of saliva in oral health is clearly demonstrated in situations in which saliva production is impaired, such as in autoimmune diseases, e.g., Sjögren's syndrome, or in patients receiving radiation treatment for head and neck cancer.[2-5]

Xerostomia or dry mouth is a condition frequently encountered in dental practices.[6,7] The most common cause is the use of systemic medications that reduce saliva production.[8,9] There is evidence that as the number of medications increases, a corresponding decrease in saliva output occurs, placing the elderly population, who are usually more medicated, at high risk for xerostomia.[8,10,11] It is also well recognized that diminished salivary flow leads to difficulty in physiologic processes, such as mastication, swallowing, and speech,[12] and can affect the quality of life as the result of problems related to the retention of dentures, infection with oral bacteria and yeast, development and progression of dental caries, and alterations in taste.[2,13]

Whole saliva is derived primarily from secretions of three pairs of major salivary glands, the parotid, submandibular, and sublingual glands, with a much smaller contribution from minor salivary glands and gingival crevicular fluid. Under resting conditions, which are prevalent in

doi: 10.1902/jop.2008.070566

the oral cavity for most of the 24-hour period, submandibular glands are the major contributors to the volume of whole saliva (65%).[6] Chronic xerostomia has a debilitating effect on the integrity of the hard and soft tissues of the mouth, and increasing evidence indicates that the submandibular glands exert regulatory influences on immune/inflammatory reactions.[14] The spatial relationship between molars and the margin of the alveolar bone is greatly affected by the continuous remodeling and rebuilding processes in the alveolar bone[15] and by periodontitis, an inflammatory periodontal disease that results in alveolar bone resorption and soft tissue attachment loss.[16] To analyze the importance of submandibular/sublingual glands in maintaining healthy periodontal conditions, we assessed how the absence of submandibular endocrine and exocrine secretions, after submandibular–sublingual salivary gland complex ablation (Sx), affected periodontal bone loss in rats submitted or not to ligature-induced experimental periodontal disease (EP).

MATERIALS AND METHODS

Fifty-four adult male Wistar rats with an initial body weight of 230 to 250 g were randomly divided into two groups, experimental (Sx) and control (sham), and were maintained on standard chow pellets‖ and tap water *ad libitum*. Housing conditions were as follows: galvanized wire cages; five animals per cage; temperature: 22°C to 24°C; humidity: 52% to 56%; and 12-hour light/dark cycles.

All experiments were performed following the National Institutes of Health guidelines for the care and use of laboratory animals (NIH 85-23, revised in 1985), and protocols were approved by the Ethical Commission of the Faculty of Dentistry, University of Buenos Aires.

Experimental Protocol

Rats were submitted to Sx or sham operations on day 0 (27 animals per group). Two weeks later, unilateral EP was induced on the right mandibular first molars of Sx and sham animals. The contralateral first molars (not ligated) of both groups (sham and Sx) were used as untreated controls. Animals were sacrificed by ether overdose 1 week later. Hemimandibles, once extracted and defleshed, were fixed in formalin for histomorphometry evaluation of interradicular bone (12 per group) or were stained with 1% aqueous methylene blue for microscopic determination of vestibular and lingual bone loss (15 per group).

Submandibulectomy

On day 0, the experimental group underwent bilateral Sx under light ether anesthesia. A midline incision of 15 mm was made through the skin and fascia of the ventral surface of the neck, the excretory ducts and main blood vessels were tied off, and the submandibular–sublingual salivary complexes were dissected free from their supporting connective tissue and removed. They were excised with preservation of the surrounding neural structures, including the marginal mandibular branch of the facial nerve and the hypoglossal and facial nerves. The other group was subjected to a sham operation that involved full exposure of the glands and subsequent incision closure with a silk thread as in Sx animals.

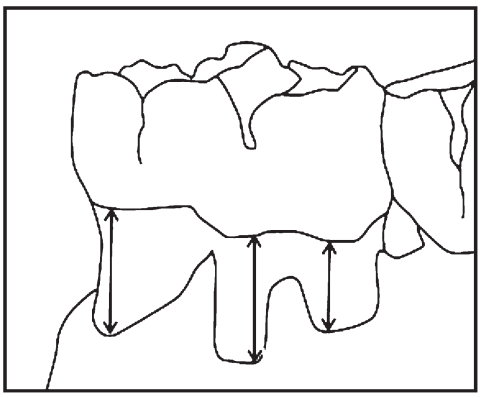

Figure 1.
Distance method: scheme of mandible and section of first molars. Three distances (arrows), which were measured on lingual and vestibular surfaces from the CEJ to the most apical area of the AC, are shown.

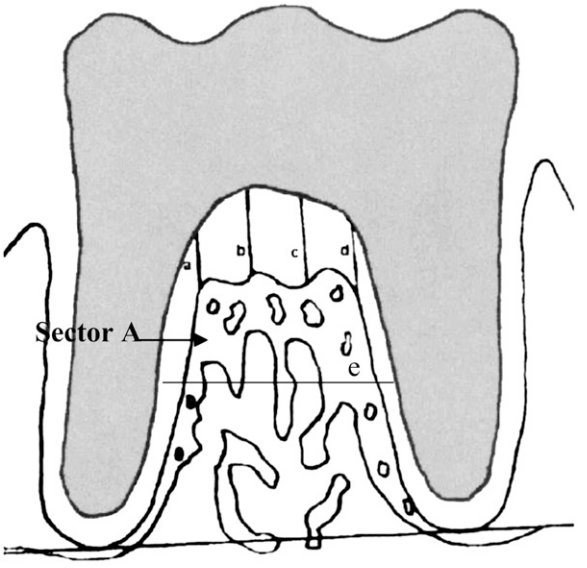

Figure 2.
Studied area of the interradicular bone: lines a through d indicate measurement points, and horizontal line e divides the interradicular bone area into two hemi-sections (Sector A = superior half). To standardize the measured area, a line was drawn tangential to the most apical area of the roots.

‖ Alimento balanceado Cooperación, Asociación de Cooperativas Argentinas, San Nicolás-Buenos Aires, Argentina.

Periodontal Disease

Two weeks after the onset of the experiment, animals were anesthetized with intraperitoneal administration of 2% xylazine hydrochloride¶ (5 mg/kg body weight) and 5% ketamine hydrochloride# (50 mg/kg body weight), and EP was induced by placing a cotton thread ligature around the neck of the lower right first molar.[17] The cotton thread ligature was pushed into the gingival sulcus and was left in place for 7 days until sacrifice. The contralateral lower first molar without treatment (no ligature) was used as a control.

Microscopic Examination of Periodontal Bone Loss: Distance Method

Immediately after sacrifice, hemimandibles were resected, defleshed, and stained with 1% aqueous methylene blue to delineate the cemento-enamel junction (CEJ) and the alveolar crest (AC). A stereomicroscope** and a digital caliper†† were used to measure three lingual and three vestibular distances (mesial, central, and distal) from the CEJ to the most apical area of the AC[18] (Fig. 1). Repeated determinations for this method yielded a relative coefficient of variation of 2%. The mean of the three recordings for each tooth surface was used as a measure of the total lingual and vestibular bone loss.

Histomorphometric Evaluation

Hemimandibles were fixed in formalin buffer immediately after resection. They were decalcified in 10% EDTA, pH 7, for 25 days. After this period, hemimandibles were dehydrated with ethanol and clarified with xylol. Finally, the sector containing the three lower molars of each decalcified hemimandible was embedded in paraffin at 56°C to 58°C. Under a stereomicroscope‡‡ and using a microtome,§§ mesio-distally oriented sections of each lower first molar were obtained from paraffin blocks. Sections 7 μm in width were stained with hematoxylin and eosin, and histomorphometric evaluation was performed on digitized microphotographs using imaging software.‖‖

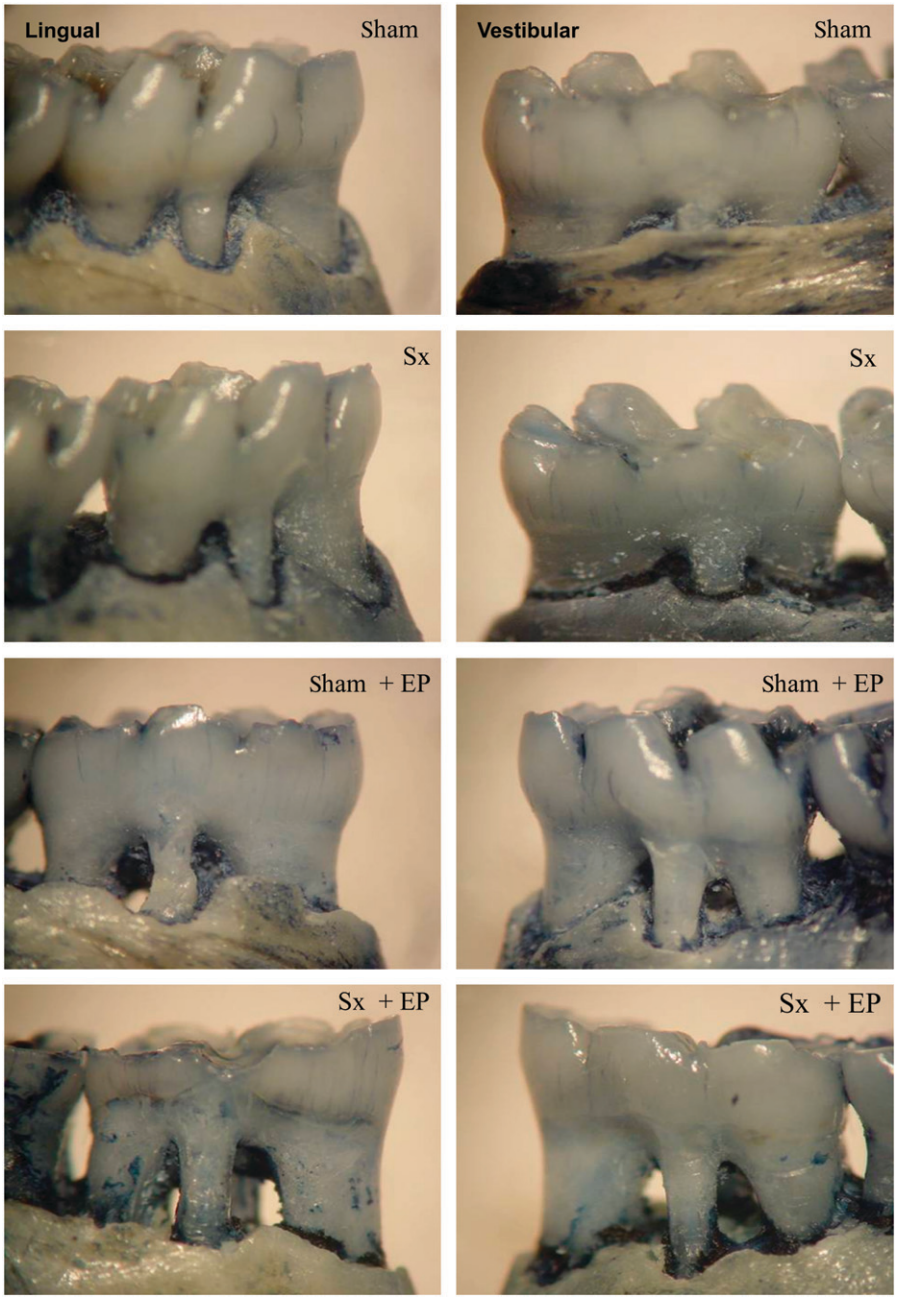

Figure 3.
Distance method: photographs of mandibles showing lingual (left column) and vestibular (right column) sections of lower first molars.

The following static parameters were evaluated on the total interradicular bone: 1) bone volume (BV)/total volume (TV) (%) = fraction of TV corresponding to

¶ König Laboratories, Buenos Aires, Argentina.
# Holliday-Scott, Buenos Aires, Argentina.
** Stemi DV4 Stereomicroscope, Carl Zeiss Microlmaging, Göttingen, Germany.
†† Digital caliper, Digimess, Geneva, Switzerland.
‡‡ Stemi DV4 Stereomicroscope, Carl Zeiss Microlmaging.
§§ Jung AG, Heidelberg, Germany.
‖‖ Image Tool, University of Texas Health Science Center at San Antonio, San Antonio, TX.

bone tissue; total volume was taken as bone tissue plus bone marrow and periodontal ligament; 2) BV/TV (%) of section A (superior half of interradicular bone) (Fig. 2); and 3) height of the periodontal ligament (in micrometers); to measure the height of the periodontal ligament, four equidistant points were marked on the AC of the interradicular bone, and a line was drawn from each of the points to the bone (Fig. 2). The length of the lines was measured, and the mean value was calculated to obtain the height of the periodontal ligament of each section.[19]

Statistical Analysis

Results were analyzed statistically by the non-parametric Kruskal-Wallis and Dunn post hoc tests using a statistical program.¶¶ The level of significance was set at 0.05 and expressed as mean ± SEM (n).

RESULTS

The measures of lingual and vestibular bone loss, as distances between the CEJ and AC, revealed greater lingual than vestibular bone loss in sham and Sx animals (Figs. 3 and 4). In the unligated molars, greater bone loss was observed on lingual surfaces of Sx animals compared to sham animals (P <0.05; Fig. 4). The presence of the cotton thread for 7 days brought about a significant increase in lingual and vestibular bone loss (P<0.001). In addition, the lingual bone loss observed in EP molars of Sx animals was greater than in sham animals (P <0.05; Figs. 3 and 4).

Histomorphometric evaluation of interradicular bone revealed significant bone resorption in EP molars (P<0.05 and P<0.001 compared to the unligated control in sham and Sx animals, respectively) and no significant effect of Sx on molars with or without EP (Figs. 5 and 6).

DISCUSSION

The results suggest that the submandibular/sublingual glands are relevant in maintaining healthy periodontal conditions. Sx in rats increased lingual and vestibular bone loss as well as the lingual bone loss induced by EP, without affecting interradicular bone. The lingual alveolar bone loss was more pronounced than the vestibular bone loss in sham and Sx animals. Conversely, the percentage of bone loss induced by EP was greater on the vestibular surface. These results agree with the study of Ariji et al.,[20] which demonstrated that the cortical plate changes induced by odontogenic infection were seen more frequently on the vestibular side than on the lingual side. The observation that molars with EP exhibited greater alveolar lingual loss in Sx animals than in sham animals suggests a local protective effect of the gland complex.

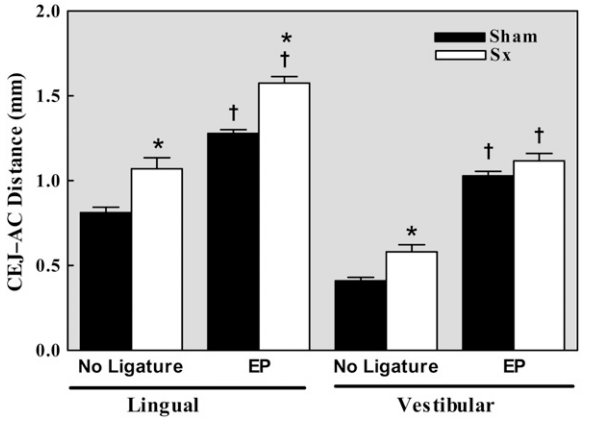

Figure 4.
*Effect of Sx and/or 1-week EP on CEJ–AC distances of lingual and vestibular surfaces. Unilateral EP was induced on the lower right first molars. The contralateral side (no ligament) served as a control. The means ± SEM (N = 15) are shown. *P <0.05 compared to its corresponding sham; †P <0.001 compared to the contralateral side with no ligature.*

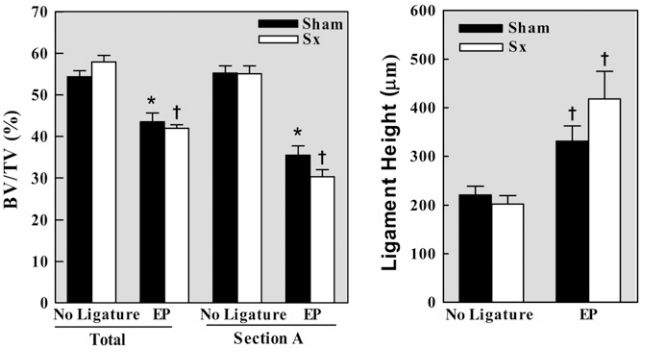

Figure 5.
*Effect of Sx and/or 1-week EP on static histomorphometry of interradicular bone. Total interradicular bone volume, bone volume of section A (superior half section of interradicular bone), and ligament height are shown. Unilateral EP was induced on the right mandibular first molars. The contralateral side (no ligament) served as control. The means ± SEM (N = 12) are shown. *P <0.05 compared to its corresponding sham; †P <0.001 compared to the contralateral side with no ligature.*

The submandibular gland produces serous, mucin-rich saliva that coats the oral tissues. Mucins represent the major organic component of submandibular/sublingual saliva. Their viscoelastic properties provide lubrication, and their high degree of glycosylation and potential for hydration prevent desiccation. As important components of the acquired pellicle and plaque matrix, mucins may also bind to toxins, agglutinate bacteria, and interact with host cells.[21-23] A reduction in the output of mucins by Sx may increase mechanical forces and induce the loss of

¶¶ GraphPad Software, San Diego, CA.

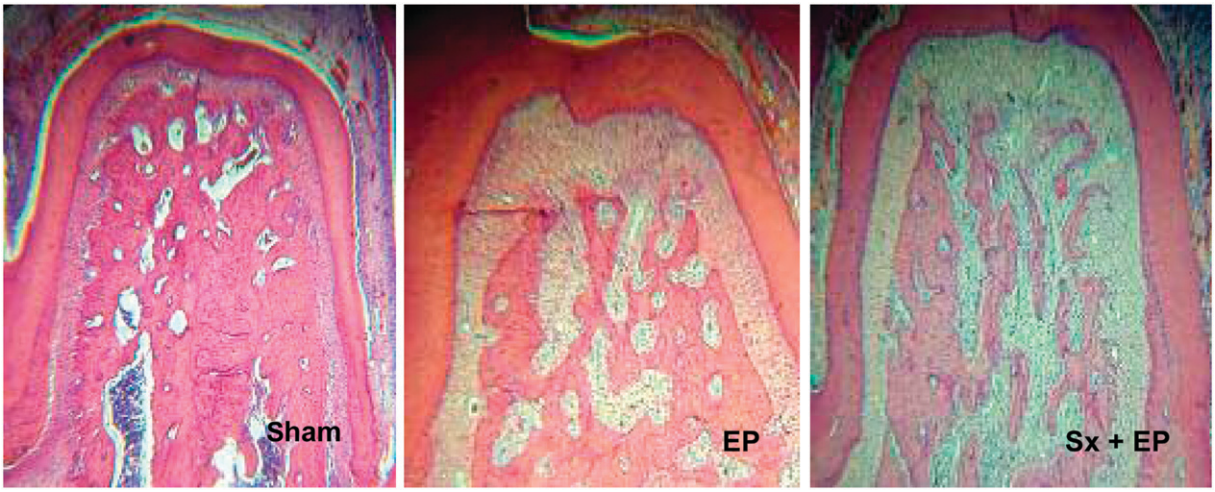

Figure 6.
Microphotographs showing interradicular bone. Sham, not ligated (left), sham + EP (middle): marked bone loss caused by periodontitis can be observed; Sx + EP (right): bone resorption similar to that in EP (middle). (Hematoxylin and eosin; original magnification ×50.)

supporting soft tissue, which, in turn, lead to loss of marginal alveolar bone as described herein.

Periodontitis is characterized by inflammation of the supporting tissues of the teeth and periodontal pocket formation that result in alveolar bone resorption and soft tissue attachment loss. In that sense, it has been suggested that the submandibular gland functions as a key regulatory organ in the oral neuroimmunoregulatory network.[14] The submandibular gland produces, among others, nerve growth factor, epidermal growth factor, transforming growth factor-beta, and kallikreins, which are secreted into the saliva and the bloodstream and affect immune and mucosal tissue. Submandibular secretions enable the gland to exert regulatory influences on immune/inflammatory reactions.[14] The submandibular/sublingual complex also produces antimicrobial proteins and secretory immunoglobulin A antibodies that are essential factors in the mucosal host defense. Suppression of all these secretions by Sx might alter the mucosal immune/inflammatory response and the regeneration and healing of periodontal support tissues, accounting for, at least in part, the negative effects of Sx on alveolar bone described herein.[14]

In situations in which saliva production is impaired, such as in autoimmune diseases like Sjögren's disease, a higher risk for periodontitis with higher plaque index scores, increased alveolar bone loss, and increased CEJ–AC distance have been reported.[24] However, the occurrence of periodontal disease in Sjögren's disease is still controversial. The health status of the gingival and periodontal tissues of these patients has also been described as similar to controls[25] or with only slight differences.[25,26] It is important to keep in mind that periodontitis is a multifactorial disease and, in most cases, a disease with a chronic progression. Exposure to factors that contribute to periodontitis occurs over a long period, so that, at the time of diagnosis, it may be difficult to identify and evaluate what cofactors have contributed to its development.[27]

Our results indicated that the absence of submandibular secretions, exocrine and endocrine, have a direct deleterious effect on the periodontal tissues, which may increase the risk for gingival and periodontal pathologies. Such observations can have relevance, taking into consideration that the single most common side effect of many drugs is dry mouth[6,9,13] and the evidence that as the number of medications per individual increases, there is a corresponding decrease in saliva output.[10] Possibly, the increased incidence of diminished submandibular secretion in elderly patients favors that chronic diseases, such as periodontitis, are more prevalent. In this respect, the present observations of greater EP-induced lingual alveolar bone loss in Sx animals than in sham animals might be relevant.

No effect of Sx on periodontal disease was observed at the level of the interradicular bone; interradicular bone loss represents an advanced stage in the development of periodontitis in relation to marginal attachment bone loss. However, it is evident that rapid loss of the alveolar cortical plate induced by Sx is causal to progression to a more complex stage in the evolution of the disease.

How the removal of glands and their endocrine and endocrine secretions affect alveolar bone deserve further investigation. Sx might induce hyposalia, and/

or changes in salivary composition, and/or plaque accumulation on teeth, and/or alter the mucosal immune/inflammatory responses and/or calcium metabolism.[28] Although the mechanisms remain unknown, our results emphasize the importance of submandibular saliva in maintaining a healthy oral environment and indicate that Sx has a deleterious effect on periodontal tissues, particularly marginal alveolar bone.

ACKNOWLEDGMENTS

The authors acknowledge the collaboration of Ricardo Orzuza, animal laboratory technician, Department of Biochemistry, Faculty of Dentistry, University of Buenos Aires, and Ana María Gomez, technical assistant, Department of Histology, Faculty of Dentistry, University of Buenos Aires. This investigation was supported by grant O 025 from the University of Buenos Aires. The authors report no conflicts of interest related to this study.

REFERENCES

1. Dodds MW, Johnson DA, Yeh CK. Health benefits of saliva: A review. *J Dent* 2005;33:223-233.
2. Fox PC. Management of dry mouth. *Dent Clin North Am* 1997;41:863-875.
3. Sreebny LM. Saliva in health and disease: An appraisal and update. *Int Dent J* 2000;50:140-161.
4. Al-Hashimi I. The management of Sjogren's syndrome in dental practice. *J Am Dent Assoc* 2001;132:1409-1417.
5. Valdez IH, Atkinson JC, Ship JA, Fox PC. Major salivary gland function in patients with radiation-induced xerostomia: Flow rates and sialochemistry. *Int J Radiat Oncol Biol Phys* 1993;25:41-47.
6. Dawes C. Factors influencing salivary flow rate and composition. In: Edgar WM, O'Mullane DM, eds. *Saliva and Oral Health*, 2nd ed. London: British Dental Association; 1996:27-41.
7. Cassolato SF, Turnbull RS. Xerostomia: Clinical aspects and treatment. *Gerodontology* 2003;20:64-77.
8. Navazesh M, Brightman VJ, Pogoda JM. Relationship of medical status, medications, and salivary flow rates in adults of different ages. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1996;81:172-176.
9. Wynn RL, Meiller TF. Drugs and dry mouth. *Gen Dent* 2001;49:10-14.
10. Gupta A, Epstein JB, Sroussi H. Hyposalivation in elderly patients. *J Can Dent Assoc* 2006;72:841-846.
11. Martin RE. Management of dry mouth in elderly patients. *J Gt Houst Dent Soc* 1994;66:25-28.
12. Tabak LA. In defense of the oral cavity: Structure, biosynthesis and function of salivary mucins. *Annu Rev Physiol* 1995;57:547-564.
13. Sreebny LM, Schwartz SS. A reference guide to drugs and dry mouth – 2nd edition. *Gerodontology* 1997;14:33-47.
14. Sabbadini E, Berczi I. The submandibular gland: A key organ in the neuro-immuno-regulatory network? *Neuroimmunomodulation* 1995;2:184-202.
15. Hoffman MM, Schour I. Quantitative studies in the development of the rat molar. II. Alveolar bone, cementum and eruption. *Am J Orthod* 1940;26:854-874.
16. Page RC, Schroeder HE. Pathogenesis of inflammatory periodontal disease. A summary of current work. *Lab Invest* 1976;34:235-249.
17. Goya JA, Paez HA, Mandalunis PM. Effect of topical administration of monosodium olpadronate on experimental periodontitis in rats. *J Periodontol* 2006;77:1-6.
18. Crawford JM, Taubman MA, Smith DJ. The natural history of periodontal bone loss in germfree and gnotobiotic rats infected with periodontopathic microorganisms. *J Periodontal Res* 1978;13:316-325.
19. Mandalunis PM, Steimetz T, Castiglione JL, Ubios AM. Alveolar bone response in an experimental model of renal failure and periodontal disease: A histomorphometric and histochemical study. *J Periodontol* 2003;74:1803-1807.
20. Ariji Y, Obayashi N, Goto M, et al. Roots of the maxillary first and second molars in horizontal relation to alveolar cortical plates and maxillary sinus: Computed tomography assessment for infection spread. *Clin Oral Investig* 2006;10:35-41.
21. Nieuw Amerongen AV, Oderkerk CH, Driessen AA. Role of mucins from human whole saliva in the protection of tooth enamel against demineralization in vitro. *Caries Res* 1987;21:297-309.
22. Slomiany BL, Murty VL, Piotrowski J, Slomiany A. Salivary mucins in oral mucosal defense. *Gen Pharmacol* 1996;27:761-771.
23. Baughan LW, Robertello FJ, Sarrett DC, Denny PA, Denny PC. Salivary mucin as related to oral *Streptococcus mutans* in elderly people. *Oral Microbiol Immunol* 2000;15:10-14.
24. Najera MP, al-Hashimi I, Plemons JM, et al. Prevalence of periodontal disease in patients with Sjögren's syndrome. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1997;83:453-457.
25. Schiødt M, Christensen LB, Petersen PE, Thorn JJ. Periodontal disease in primary Sjögren's syndrome. *Oral Dis* 2001;7:106-108.
26. Jorkjend L, Johansson A, Johansson AK, Bergenholtz A. Periodontitis, caries and salivary factors in Sjögren's syndrome patients compared to sex- and age-matched controls. *J Oral Rehabil* 2003;30:369-378.
27. Persson GR. What has ageing to do with periodontal health and disease? *Int Dent J* 2006;56:240-249.
28. Feng YS, Wase AW. Sialoadenectomy and the metabolism of calcium-45 in bone and soft tissues of the mouse. *Nature* 1956;178:1229-1230.

Correspondence: Dr. María Irene Vacas, Department of Biochemistry, Faculty of Dentistry, University of Buenos Aires, M.T. de Alvear 2142, Piso 12 B, 1122 Buenos Aires, Argentina. Fax: 54-114-511-8124; e-mail: vacasmirene@hotmail.com.

Submitted October 31, 2007; accepted for publication December 12, 2007.