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Research Article

Ultrasurface Morphological Changes in the Rat Tongue Posterior One-Third Exposed to Passive Smoke

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Abstract

Introduction: Passive smoke or second hand smoke is also called environmental tobacco smoke; it is the result of spontaneous cigarette burning as well as the smoke exhaled by active smokers. Passive smoke has found to cause health problems as active smoke.

Aim of the work: To investigate the harmful effects of passive smoke on the posterior one-third of the rat tongue by scanning electron microscope (SEM).

Materials and Methods: Thirty male albino rats were used and divided randomly into 3 groups. Group 1 (n=10) considered as control and not exposed to smoking. Group 2 rats (n=10) were exposed for 40 days to passive cigarette smoke, 4 cigarettes twice daily in closed smoking device. Meanwhile, group 3 rats (n=10) were handled as group 2 and instead exposed to the passive smoke for 60 days. The animals were euthanized at the end of the experimental periods, the posterior one-third of the tongues were dissected. The specimens for H&E stain were fixed immediately in 10% formaldehyde while the specimens for SEM investigation were fixed immediately in 2.5% buffered glutaraldehyde then prepared for histological study.

Results: Group 1 revealed normal appearance of conical, bifurcated or trifurcated filiform papillae with few numbers of cocci on the dorsal epithelial surface. The ventral surface showed normal appearance of the superficial epithelial cells. Group 2 revealed disfigurement of filiform papillae with areas of their fusion and adhesion,numerous grouping of bacteria while the ventral surface showed abnormal surface architecture with desquamation and wrinkled appearance or hyperkeratosis. Group 3 revealed disfigurement of the filiform papillae with numerous polymicrobial grouping intermingled with lymphocytes and macrophages. The ventral surface showed folds,hyperkeratosis and surface epithelial fissures.

Conclusion: Passive smoke is associated with epithelial pathological changes of the rat tongue epithelium with the carriage of pathogenic species of bacteria on tongue surface.

Recommendation: Cigarette smoke should be avoided and prevented to protect smokers and non-smokers.

Keywords: *Passive smoke; Rat tongue posterior one-third; Dorsal surface; Ventral surface; Filiform papillae*

Introduction

Smoking is one of the leading causes of avoidable death globally [1]. It is not only a problem for smokers, but also for the surrounding nonsmokers, which is called passive smoking that exposes non-smokers to most of the toxic chemicals, gases, and particles that smokers inhale directly with tobacco smoke [2]. Tobacco smoke contains about 200 toxic substances, and 69 of these have been identified as carcinogens [3]. It is evident that smoking has many adverse effects on oral cavity such as oral cancer, periodontal diseases, cleft lip, cleft palate, alveolar bone loss, and black hairy tongue [4-6], leukoplakia, oral candidiasis, staining of teeth, decrease wound healing, and reduction of the capability to taste and smell [7]. Passive smoking causes several diseases as well as leads to exacerbations of many diseases

of major public health importance. In children, passive smoking was found to increase the susceptibility of middle ear infections, lower respiratory tract infections, asthma and sudden infant death syndrome. While in adults, passive smoking increases the risk of cardiovascular diseases, asthma, and lung cancer [8].

The tongue is the most accessible organ of the oral cavity. Traditionally, tongue lesions have been considered disorders of primary concern regarding oral and general health [9]. The tongue is actually a muscular complex organ covered by epithelium and has different functions like swallowing, suckling, phonation, jaw movement, and perception of sensations including taste, thermal changes, pain stimuli and general sensations [10]. Such functions can be affected by the changes in the oral environment and the extreme alterations in thermal, mechanical and microbial factors [11].

Abnormalities of the tongue can present a diagnostic and therapeutic dilemma for physicians [12]. Tobacco smoking is considered as one of the most important risk factors leading to the development of oral mucosal lesions as leukoplakia and black hairy tongue [13]. Sparse researches regarding the electron microscopic examination of the effects of passive smoking on tongue epithelium are present and controversy remains among the studies on the effects of passive smoking on the tongue. Thus, the purpose of the present study aimed to evaluate the effects of passive smoke on the tongue surface. The null hypothesis of this animal study was that there was no ultrasurface morphological changes in the posterior one-third of the rat tongue exposed to passive smoke.

Materials and Methods

The study was conducted under approval from the Animal Welfare Committee of Mansoura University. All animals were fed standard pellet diet and water in an animal house under standard laboratory conditions. Thirty adult male white albino rats, weighting 150 to 200 g were selected and divided randomly into 3 groups. Group 1 (n = 10) considered as control and not exposed to smoking. Group 2 rats (n = 10) were exposed for 40 days to passive cigarette smoke, 4 cigarettes (Cleopatra, Eastern Company, Cairo, Egypt), twice daily in closed smoking device [14]. Meanwhile, group 3 rats (n = 10) were handled as group 2 and instead exposed to the passive smoke for 60 days. The animals were euthanized at the end of the experimental periods The posterior one-third of the tongues were dissected and the specimens for H&E stain was fixed immediately in 10% formaldehyde The specimens for scanning electron microscopic (SEM) investigation were fixed in 2.5% buffered glutaraldehyde in 0.1M (mol) phosphate buffered saline (PBS), pH 7.4 at 4^0C (2 hours).

Regarding SEM and after fixation, the specimens were washed 3 times with PBS (10 min each). Post fixation in 1% osmic acid (90 min) was done. Washing 3 time with PBS (10 min each), then dehydrated with ascending series of ethyl alcohol (30%, 50%, 70%, 90% and absolute alcohol) and infiltrated with acetone. The samples were dried with the critical point drying machine (Structure Probe Inc, West Chester, PA, USA) using liquid CO_2. Specimens were mounted with aluminum stubs, coated with gold in TMVas/sputter module (Structure Probe Inc, West Chester, PA, USA). SEM examination was done at the Electron Microscopic Unit, Faculty of Agriculture, Mansoura University. SEM (JEOL JSM-6510LV low vacuum) is a high-performance SEM for fast characterization and imaging of fine structures with a high resolution of 3.0 nm at 30 kV.

Smoking device: It is a closed glass container with sliding glass slap at its top end for the ventilation and handling of rats before and after exposure, there was a hole at one side by which main stream of cigarette enters the device.

Results

Haematoxylin and eosin histological results

At the two examination periods, group 1 specimens showed normal tongue posterior one-third histological structure for the dorsal, ventral surfaces and the tissues in-between. The dorsal surface revealed normal cylindrical or conical curved shapes with regular sizes and orientation of the filiform papillae. Filiform papillae were covered with keratinized stratified squamous epithelium and had a core of connective tissue papilla. The lamina propria adjacent to the basal layer of epithelium and thick layers of striated muscles running in different planes. No lymphatic follicles detected in the posterior one-third (Figure 1A). Meanwhile, the ventral surface showed keratinized

stratified squamous epithelium, underlined by lamina propria and thick layers of skeletal muscle fibers that are grouped into bundles separated by connective tissue (Figure 1C). After sixty days of exposure to passive smoke, the dorsal surface of the posterior one-third for group 3 showed disfigurement of filiform papillae, some appeared shorter with eroded tips while others had hyperkeratosis and adherence of the filiform papillae (Figure 1B). The ventral surface after sixty days showed hyperkeratosis (Figure 1D).

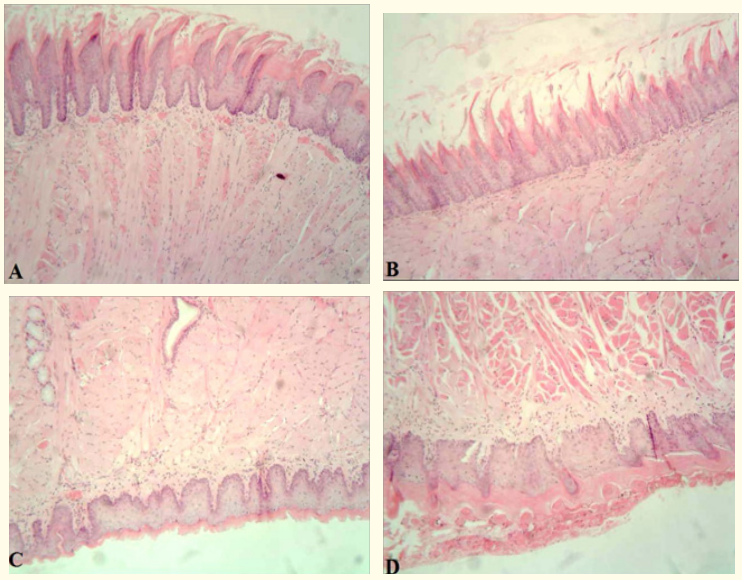
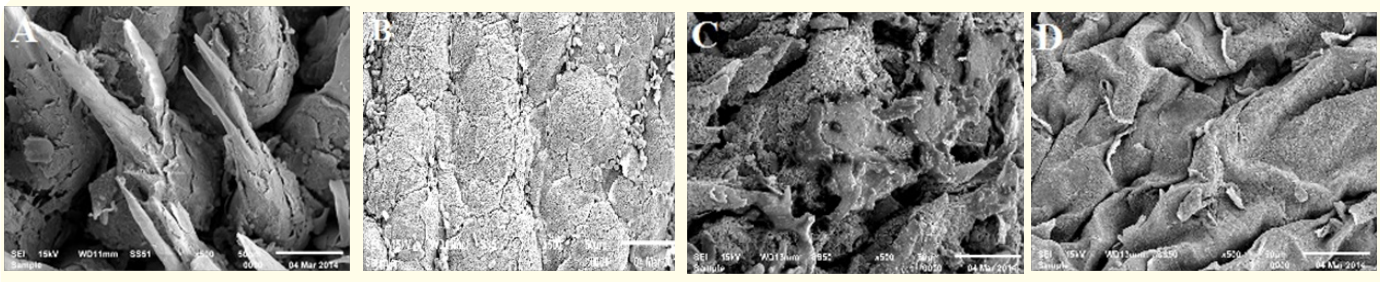


Figure 1: *Photomicroscopic picture of the posterior one-thrid of the rat tongue dorsal surface in group 1 showing normal architecture of filiform papillae, keratinized stratified squamous epithelium, lamina propria and thick muscle layers (A) while group 3 sections showing disfigurement of filiform papillae with abnormal keratinization and adherence to each other (B). The ventral surface of the rat tongue in group 1 showing normal keratinized stratified squamous epithelium, lamina propria and thick muscle layers (C) while group 3 sections showing hyperkeratosis (D) (H&E 100x).*

Scanning electron microscope results

Group 1 revealed normal appearance of large or small bifurcated and trifurcated filiform papillae in the posterior area of rat tongue. Also few numbers of cocci was seen on the epithelial surface (Figure 2A). The ventral surface showed normal appearance of epithelial cells with red blood corpuscles on the surface epithelium (Figure 2B). Group 2 revealed disfigurement and areas of fusion of filiform papillae (Figure 2C). The ventral surface showed abnormal architecture with wrinkled appearance and desquamation or hyperkeratosis (Figure 2D). Numerous grouping of bacteria (Figure 2E, 2F, 2G) Group 3 revealed disfigurement of filiform papillae with numerous polymicrobial grouping of cocci intermingled with lymphocytes and macrophages (Figures 2H, 2I,2J). The ventral surface revealed epithelial fissures, deposits,.folds, desquamation and fissures (Figure 2K and 2L).



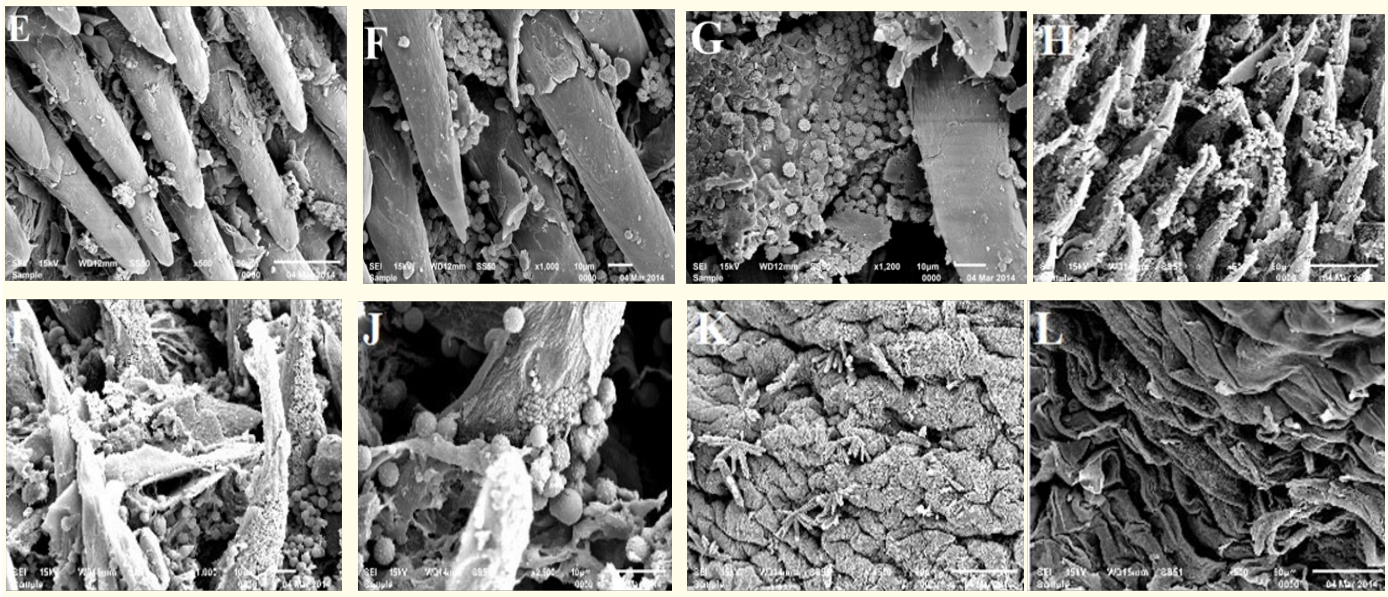


Figure 2: *Scanning electron micrographs of the dorsal surface of the tongue of group 1 showing bifurcated and trifurcated filiform papillae with some cocci between the papillae (A) and intact surface epithelialization with aggregation of RBCs on the ventral surface of the tongue epithelium (B). The dorsal surface of the tongue in group 2 showing area of desquamation with the disfigurement and adherence of the filiform papillae (C) while the ventral surface showing desquamated and irregular surface epithelium with loss of its architecture (D) and numerous grouping of bacteria (E) (SEM x500). Figures F and G are higher magnification for Figure E, F with x1000 and G with x1200. Scanning electron micrograph of the dorsal surface of the tongue of group 3 showing numerous grouping of bacteria on the surface of the filiform papillae (H, SEM, 500x), disfigurement of filiform papillae (I, SEM, 1000x), desquamation, leucocytes aggregation and grouping of bacteria (J, SEM, 2500x). The ventral surface of the tongue of group 3 showing disfigurement, desquamation, folds, deposits and fissures of epithelial layer (K, L,.SCM, 500x).*

Discussion

Passive smoke can adversely affect the health of nonsmokers of all age groups [15]. Light microscopic study of the posterior one-third of the rat tongue revealed absence of lymphatic tissue in both the control and experimental groups. These findings were in consistent with the Casteleyn., *et al*. [16] who concluded that proper tonsils are absent in the rat, and pigeon. SEM results for the tongue posterior one-third revealed filiform papillae in the form of conical, bifurcated or trifurcated shapes with uniform distribution in the control group. This is consistent with Nasr., *et al*. [17] who found that bifurcated or trifurcated papillae are present in posterior tongue areas with a few fungiform papillae were detected mostly in the apex. One circumvallate papilla with large macroscopic size was detected in front of the root. Keratinized stratified squamous epithelium was found to cover the dorsal surface of the mucosa and thinner keratin on the ventral surface [18,19]. In the present study, cocci were seen in the control group and this is consistent with Watanabe., *et al*. [20] who detected different groups of cocci and staphylococci between filiform and fungiform papillae by high-resolution SEM images. In control group, scanning electron micrograph of the inferior surface of the tongue revealed an intact epithelial layers with red blood cells seen on the surface and this can be attributed to immediate fixation without washing.

Experimental groups revealed the same types of papillae with adherence and disfigurement with change in keratinization. Hyperkeratinization was seen in the inferior surface of the tongue and this is consistent with the several histologic studies [21-25] that reported the occurrence of various lesions in the airways of rats exposed to cigarette smoke as inflammation, hyperkeratosis, acanthosis, leukoplakias, erythroplakias, dysplasia, and carcinoma. However, examination using SEM is rare.

Group 2 revealed disfigurement, desquamation of epithelial layer, thickened, folded and irregular epithelial surface or hyperkeratinization. Similar results were observed by Martin [25], who examined the surface of the tongue, pharynx, and larynx of animals exposed to tobacco and found that the filiform tongue papillae of the tobacco group were irregularly displayed, flattened, and adhered to each other. Reichart and Althoff [26] detected that leukoplakias and dysplasias of the squamous epithelium showed hyperkeratosis, the folding irregularities, and the increased superficial peeling.

Another study observed greater destruction and disorganization of tongue papillae when exposing hamsters to cigarette smoke [27]. Transmission electron microscope examination of rats injected daily with intraperitoneal nicotine showed epithelial atrophy, cell membranes disorganization, and tissue damage after 90 days. Those authors explained that these epithelial changes may predispose the epithelium of the oral mucosa to be affected by the other carcinogenic agents present in the cigarette [23].

Group 2 and 3 showed adherence of numerous bacteria on the outer surface of epithelial cells, were found to colonize on the dorsal surface of the tongue. These results are consistent with other studies which showed increased number of *S. mutans* with the use of 0.25 to 8 mg/ml nicotine [28,29] and it seems that the growth of S. sanguis and *S. mutans* is accelerated in the vicinity of cigarette smoke [30]. Another study [31] found that cigarette tobacco smoke can interfere with the adhesion and biofilm formation of these microorganisms to various orthodontic materials. An in vitro study by Barão [32] found that the growth of cultivable S. sangius biofilm to commercially-pure titanium is enhanced by nicotine and cotinine at physiological levels in the saliva and crevicular fluid of smokers. Moreover, Sakki., *et al*. [33] showed that smoking was strongly related to increased numbers of lactobacilli and yeast.

The polymicrobial growth effect of nicotine on the oral microbiota and especially streptococcus bacteria can be explained by multiple mechanisms. It was found that Haemophilus influenza might use nicotine as a nutrient material, inducing the enhancement in biofilm growth [34]. Other microbes in the environment use the nicotine as a source of nitrogen, carbon and energy for their development [35]. When S mutans biofilm is exposed to nicotine, increased formation of extracellular polysaccharide substance (EPS) was shown [29,32]. EPS is also important for bacterial cell attachment and biofilm growth [36,37] and is synthesized by glucosyl transferases. Nicotine was reported to increase expression of glucosyltransferases, antigen I/II, and glucan-binding protein A as well as increase *S. mutans* metabolic activity [28].

Nicotine has been found to cause oxidative stress in human cells, and the transcriptional repressor Rex was shown to regulate the response of *S. mutans* to oxidative stress and biofilm formation. *S mutans* bacteria with deficient Rex mutant showed decreased biofilm formation, caused by the altered expression of genes responsible for carbohydrate fermentation, NAD+/NADH recycling and oxidative homeostasis [37].

Smoking inhibits fibroblasts [39] by affecting nicotinic acetylcholine receptors [40] and facilitates the intrusion of Porphyromonas gingivalis into epithelial cells [41] and human gingival fibroblasts [42]. It has been reported that substances present in tobacco smoke change the charge and other properties of oral epithelial cell surfaces, facilitating the growth of certain pathogenic bacteria [43]. In previous study there was significant increase in white blood cell count and significant decrease in glutathione level after sixty days of passive smoke exposure which is explained and confirmed by this this study [44].

So far active and passive exposure to cigarette smoke are associated with increased potentially pathogenic bacteria in both adults and children [45]. In addition to the harmful histological changes. Therefore, prevention and avoidance of smoke will not only be beneficial for the smokers but also will protect non-smokers.

Conclusion

Exposure to passive smoke is associated with epithelial pathological changes in both dorsal and ventral surface of the tongue with carriage of numerous grouping of bacteria which may lead to dangerous oral and respiratory diseases.

Recommendation

Cigarette smoke should be avoided and prevented to protect smokers and non-smokers. Further bacteriological studies are recommented to confirm and determine the spieces of pathological bacteria associated with passive smoke.

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Bibliography

1. Wipfli H and Samet JM. "Global economic and health benefits of tobacco control: part 1". *Clinical Pharmacology & Therapeutics* 86 (2009): 263-271.

2. Jamrozik K. "Estimate of deaths attributable to passive smoking among UK adults: database analysis". *BMJ* 330 (2005): 812.

3. Hoffmann I and Hoffmann D. "The changing cigarette: chemical studies and bioassays". In Tobacco: Science, policy and public health, Oxford, UK: Oxford University Press, (2010).

4. Khalili J. "Oral cancer: risk factors, prevention and diagnostic". *Experimental oncology* 30 (2008): 259-264.

5. Bánóczy J., *et al.* "Tobacco use and oral leukoplakia". *Journal of Dental Education* 65 (2001): 322-327.

6. Khor GH. "Chromosome 17 Aberration of oral squamous cell carcinoma in Malaysia". *Global Journal of Health Science* 1 (2009): 150-156.

7. Vellappally S., *et al.* "Smoking related systemic and oral diseases". *Acta Medica (Hradec Kralove).* 50 (2007): 161-166.

8. Jaakkola MS and Jaakkola JJ. "Adverse health effects of passive smoking". Duodecim 128 (2012): 1097-1106.

9. Avcu N and Kanli A. "The prevalence of tongue lesions in 5150 Turkish dental outpatients". *Oral Diseases* 9 (2003): 188-195.

10. duToi DF. "The tongue: Structure and function relevant to disease and oral health". *SADJ* 58 (2003): 375-376.

11. Rioboo-Crespo Mdel R., *et al.* "Epidemiology of the most common oral mucosal diseases in children". *Med Oral Patol Oral Cir Bucal* 10 (2005): 376-387.

12. Terai H and Shimahara M. "Atrophic tongue associated with Candida". *Journal of Oral Pathology & Medicine* 34 (2005): 397-400.

13. Raman P., *et al.* "Prevalence of tongue lesions in tobacco and non-tobacco users of OPD, SRM Dental College, Chennai: A Cross Sectional Study". *International Journal of Advanced Health Sciences* 1 (2015): 1-5.

14. Rahman I., *et al.* "Induction of gamma-glutamylcysteine synthetase by cigarette smoke is associated with AP-1 in human alveolar epithelial cells". *FEBS Letters* 396 (1996): 21-25.

15. El Batran MM., *et al.* "Passive smoking and alveolar bone density". *Australian Journal of Basic and Applied Sciences* 3 (2009): 713-719.

16. Casteleyn C., *et al.* "The tonsils revisited: review of the anatomical localization and histological characteristics of the tonsils of domestic and laboratory animals". *Clinical and Developmental Immunology* (2011): 472460.

17. Nasr ES., *et al.* "Light and scanning electron microscopic study of the dorsal lingual papillae of the rat Arvicanthis niloticus (Muridae, Rodentia)". *AmericanScience.Org* 8 (2012): 619-627.

18. Iwasaki S. "Evolution of the structure and function of the vertebrate tongue". *Journal of Anatomy* 201 (2002): 1-13.

19. Ghassemi and Cheshmi G. "Comparative histological study of tongue in two species of rat (Rattus Norvegicus& Rattus Wistar)". *CJZ* 3 (2014): 13-21.

20. Watanabe IS., *et al.* "Ultrastructure of the adhesion of bacteria to the epithelial cell membrane of three-day postnatal rat tongue mucosa: a transmission and high-resolution scanning electron microscopic study". *Brazilian Dental Journal* 18 (2007): 320-323.

21. Caldeira EJ., *et al.* "Morphological alterations in the epithelium of the oral mucosa of rats (Rattus norvegicus) submitted to long-term systemic nicotine treatment". *Archives of Oral Biology* 52 (2007): 83-89.

22. Bolton SJ., *et al.* "Characterization of the proximal airway squamous metaplasia induced by chronic tobacco smoke exposure in spontaneously hypertensive rats". *Respiratory Research* 10 (2009): 118.

23. de Oliveira Semenzati G., *et al.* "Histological and immunohistochemical study of the expression of p.53 and ki-67 proteins in the mucosa of the tongue, pharynx and larynx of rats exposed to cigarette smoke". *Inhalation Toxicology* 24 (2012): 723-731.

24. Garcia Martins RH., *et al.* "Effects to exposure of tobacco smoke and alcohol on the tongue and pharynx of rats". *Inhalation Toxicology* 24 (2012): 153-160.

25. Martins RH., *et al.* "Scanning electron microscopy of the tongue, pharynx, and larynx of rats exposed to cigarette smoke". *Journal of Voice* 28 (2014): 287-290.

26. Reichart PA Althoff J. "Oral leukoplakia: a scanning electron microscopic study of epithelial surface patterns". *International Journal of Oral and Maxillofacial Surgery* 12 (1983): 159-164.

27. Harada T., *et al.* "Oral leukoplakia and costochondral hyperplasia induced by diethylnitrosamine in hamsters exposed to cigarette smoke with or without dietary vitamin C". *Veterinary Pathology* 24 (1987): 257-264.

28. Huang R., *et al.* "Effect of nicotine on growth and metabolism of Streptococcus mutans". *European Journal of Oral Sciences* 120 (2012): 319-325.

29. Li M., *et al.* "Effect of nicotine on dual-species biofilms of Streptococcus mutans and Streptococcus sanguinis". *FEMS Microbiology Letters* 350 (2014): 125-132.

30. Zonuz AT., *et al.* "Effect of cigarette smoke exposure on the growth of Streptococcus mutans and Streptococcus sanguis: an in vitro study". *Nicotine & Tobacco Research* 10 (2008): 63-67.

31. Baboni FB., *et al.* "Influence of cigarette smoke condensate on cariogenic and candidal biofilm formation on orthodontic materials". *American Journal of Orthodontics and Dentofacial Orthopedics* 138 (2010): 427-434.

32. Barão VA., *et al.* "The role of nicotine, cotinine and caffeine on the electrochemical behaviour and bacterial colonization to cp-Ti". *Materials Science and Engineering C: Materials for Biological Applications* 56 (2015): 114-124.

33. Sakki T and Knuuttila M. "Controlled study of the association of smoking with lactobacilli, mutans streptococci and yeasts in saliva". *European Journal of Oral Sciences* 104 (1996): 619-622.

34. Roberts D and Cole P. "Effect of tobacco and nicotine on growth of Haemophilus influenzae in vitro". *Journal of Clinical Pathology* 32 (1979): 728-731.

35. Ruan A., *et al.* "Studies on biodegradation of nicotine by Arthrobacter sp. strain HF-2". *Journal of Environmental Science and Health, Part B* 41 (2006): 1159-1170.

36. Donlan RM. "Biofilms: Microbial Life on Surfaces". *Emerging Infectious Diseases journal* 8 (2002): 881-890.

37. Donlan RM and Costerton JW. "Biofilms: survival mechanisms of clinically relevant". *Clinical Microbiology Reviews* 15 (2002): 167-193.

38. Bitoun JP., *et al.* "Transcriptional repressor Rex is involved in regulation of oxidative stress response and biofilm formation by Streptococcus mutans". *FEMS Microbiology Letters* 320 (2011): 110-117.

39. Biondo-Simões Mde L., *et al.* "The influence of nicotine on the population of fibroblasts in cutaneous scars in rats". *Acta Cirurgica Brasileira* 24 (2009): 466-470.

40. Wang XJ., *et al.* "Functional expression of alpha 7 nicotinic acetylcholine receptors in human periodontal ligament fibroblasts and rat periodontal tissues". *Cell Tissue Research* 340 (2010): 347-355.

41. Cogo K., *et al.* "The effects of nicotine and cotinine on Porphyromonas gingivalis colonisation of epithelial cells. *Archives of Oral Biology* 54 (2009): 1061-1067.

42. Allam E., *et al.* "Smoking and oral health". In: Cigarette smoke soxicity-linking individual chemicals to human diseases. Bernhard D, ed. Berlin: Wiley-VCH; (2011): 257-280.

43. El Ahmer OR., *et al.* "The effect of cigarette smoke on adherence of respiratory pathogens to buccal epithelial cells". *FEMS Immunology and Medical Microbiology* 23 (1999): 27-36.

44. Rasha H AL-Serwi., *et al.* "Impact of passive smoke on tongue blood vessels, blood cell count and blood glutathione level (experimental study)". *EC Dental Science* 2.6 (2015): 423-434.

45. Sikorska-Jaroszyńska MH., *et al.* "Passive smoking as an environmental health risk factor". *Annals of Agricultural and Environmental Medicine* 19 (2012): 547-550.

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