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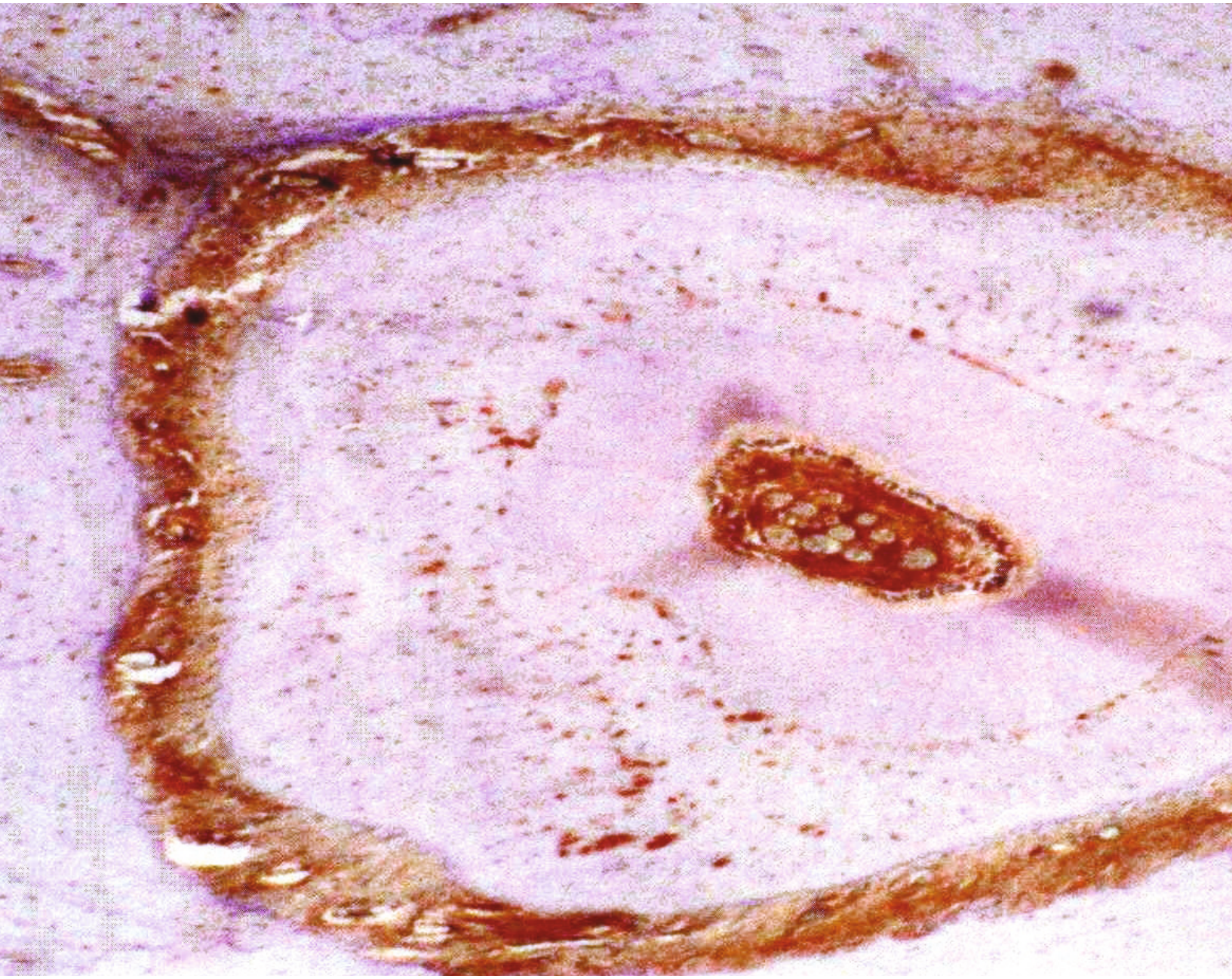

Original Article

Noise rich in low frequency components, a new comorbidity for periodontal disease? An experimental study

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Abstract:

Introduction: Exposure to noise rich in low frequency components induces abnormal proliferation of extracellular matrix and collagens. The previous studies have shown alterations in the periodontium of both humans and animals. Our objective was the evaluation of collagens I, IV and V of the periodontium of Wistar rats exposed to noise rich in low frequency components. **Materials and Methods:** 5 groups (each with 10 animals) were exposed to continuous low frequency noise (LFN). The LFN, from previously recorded white noise, frequency filtered and amplified, was applied in growing periods of 1, 3, 5, 9 and 13 weeks, in order to characterize the alterations with exposure time. A control group of ten animals was kept in silence. These animals were used in groups of 2 as aged-matched controls. After exposure, sections were obtained including teeth, alveolar bone and periodontium and observed after immunollabeling for collagens I, IV and V. **Results:** A significant increase in collagen I was observed in exposed groups ($P < 0.001$) (Kruskal-Wallis test). *Post-hoc* comparisons (Mann-Whitney test with Bonferroni correction) showed an increase in collagen I in animals exposed for 3 weeks or more ($P < 0.001$). The same test was applied to collagen V where significant differences were found when comparing control and exposed groups ($P \leq 0.004$). The *t*-test for independent samples was applied to collagen type IV where no significant differences were found ($P = 0.410$), when comparing to the control group. **Discussion:** As in other organs, we can observe fibrosis and the newly formed collagen is likely to be "nonfunctional," which could have clinical impact. **Conclusion:** Noise may constitute a new comorbidity for periodontal disease.

Key words:

Fibrosis, low frequency noise, periodontal disease, periodontium

INTRODUCTION

The non-auditory health effects of noise are long referred in the literature by many researchers.[1] Noise rich in low frequency components is known to cause abnormal proliferation of extracellular matrix and collagens, tissue fibrosis and changes in the epithelia in the absence of inflammatory processes.[2] Cardiac,[3,4] arterial and lymphatic lesions,[5,6] have been documented as well as respiratory lesions.[7] In addition, there are potential genotoxic effects and immunological changes.[8,9] Gastric and duodenal lesions have also been reported.[10]

The noise used in our study is comparable to that present in the everyday environment of industrialized nations,[1] produced by numerous sources, as airplanes, helicopters, trains, road traffic, ventilation equipment, among others.

There are few studies concerning the oral cavity and its structures related to noise effects. A study by Oliveira *et al*.[11,12] found morphologic and functional alterations in the parotid gland exposed to noise rich in low frequency components. Haskell in 1975 and Carlson and Zackrisson[13,14] in 1977 reported, in a group of aircraft pilots, alveolar bone loss strongly correlated with flight hours and high noise levels. In previous studies in the periodontium of rats our group reported morphologic alterations, disorganization, and loss of the normal architecture,[15] so periodontium, including the periodontal ligament, alveolar bone and cementum are microscopically affected.

The objective of this study was to evaluate collagens I, IV and V alterations in the periodontium of Wistar rats exposed to noise rich in low frequency components.

MATERIALS AND METHODS

Experimental protocol

We used 60 albino rats (Mus norvegicus albinus), Wistar strain adults in similar number of gender, aged 6 months, divided into 6 equal groups: One kept in a silent environment (gC) and used as aged-matched controls in groups of 2, and the remaining five exposed to continuous noise rich in low frequency components for 1 week (g1),

3 weeks (g3), 5 weeks (g5), 9 weeks (g9) and 13 weeks (g13), in order to characterize the lesions by exposure time to low frequency noise (LFN).

A computer (Compaq, USA) reproduced, continuously, a white noise previously recorded, amplified with a 200 W amplifier (QSC, USA), filtered and delivered by a subwoofer (Magnat XTC 1200, Germany), placed in front of the cages, thus creating an environment rich in low frequency components. The noise was measured with a digital spectrum analyzer (Band K 2144, USA). Spectral analysis revealed frequency bands below 500 Hz, corresponding to a sound pressure level exceeding 85 dB in the frequency bands between 20 Hz and 40 Hz as shown in previous studies.[15]

The rats were kept in cages in groups of two or three animals with no limits to their movements and were exposed to cycles of 12 h light/dark. All animals were fed with standard rat chow, had unrestrained access to water, and were treated according to the EU directive on Animal Protection for Experimental and Scientific Purposes (86/609/CE) and also according to the Portuguese laws in that regard. Ethical committee clearance was acquired for the study. Animals were sacrificed with a lethal intraperitoneal injection of ketamine (4.0-8.0 mg/Kg).

Imunollabeling

After exposure, sections were obtained including teeth, alveolar bone and periodontium, and observed after immunollabeling for collagens I, IV and V. Immunollabeling was performed by standard methods, using anticollagen I, IV and V sera (Novotec – France), specific for mouse. These immunosera stain brown and the color intensity reflects the amount of collagen present in the tissue. The antigenic recovery was carried out by enzymatic digestion with pepsin (Novotec – France), at pH between 1.5 and 2 and temperature of 37.5°C.

Statistical analysis

Statistical comparison between groups with different duration of exposure (experimental factor) for the level of brown colors in samples (endogenous or dependent variable) was performed using the Kruskal-Wallis test because the normality and homocedasticity assumptions for ANOVA one-way were validated. The color intensity of labeling was measured and analyzed with Image J software (NIH, USA). We used the pluggin "color deconvolution" to assess the amount of a given color image obtained during image capture. The images were analyzed in predefined vectors that allowed the separation of brown tones and graphic translation intensities using a histogram with a 1-225 scale.

The collected data were studied applying the *t*-test for independent samples to collagen type IV, and Kruskal-Wallis test to collagens type I and V, followed by the Mann-Whitney test with Bonferroni correction in *post-hoc* comparisons, in the cases where in the nonparametric analysis of variance (Kruskal-Wallis) detected significant differences between groups.

RESULTS

Only statistically valid groups were taken into account for the analysis.

Concerning immunolabeling for collagen I [Figures 1-3], we observed significant differences between groups ($P < 0.001$) according to the Kruskal-Wallis test. *Post-hoc* comparisons showed no significant differences between the control group (gC) and the group exposed for 1 week (g1) ($P = 0.068$). However, when compared to 3 weeks and 13 weeks the control group had levels of staining significantly lower ($P < 0.001$). There were no significant differences between g3 and g13.

Regarding collagen IV there were no differences between groups ($P = 0.410$) [Figures 4-6].

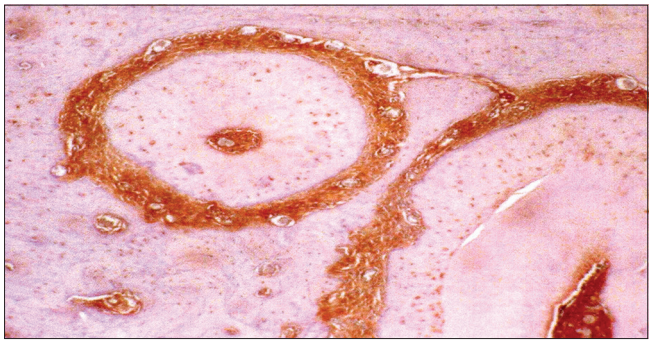

Figure 1: Periodontium imunolabeling for collagen I (collagen I, ×200). gC

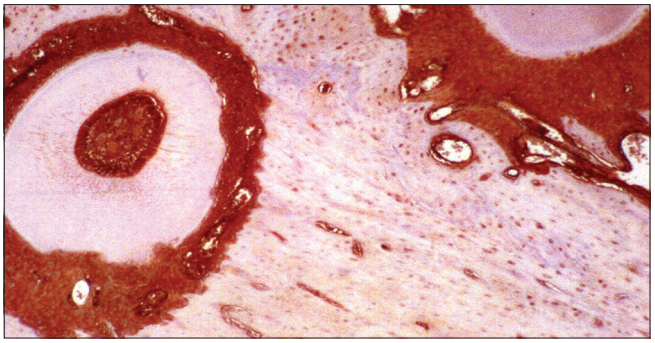

Figure 2: Periodontium imunolabeling for collagen I (collagen I, ×200). g13. The staining for collagen I (brown) increases with exposure time reaching maximum at 13 weeks exposure to low frequency noise

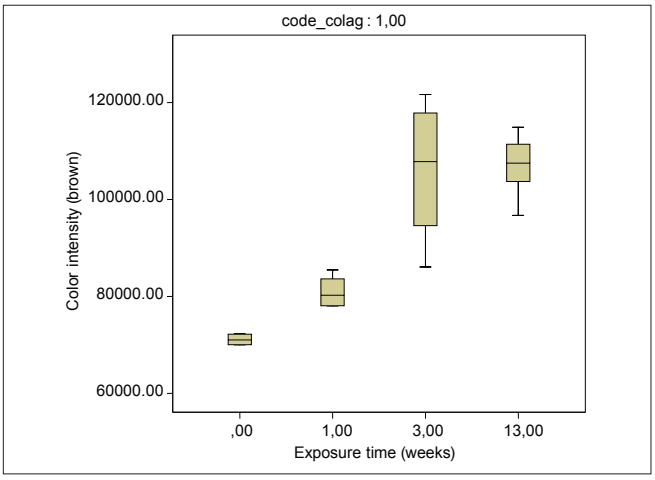

Figure 3: Immunolabeling for collagen type I. in animals exposed to low frequency noise there is an increase in immunolabeling for collagen I. There are significant differences between groups, except for g3 and g13

With respect to collagen V we found significant differences between gC and the group exposed to 13 weeks ($P \leq 0.004$) [Figures 7-9].

DISCUSSION

There is lack of knowledge concerning the effects of noise in the mouth and periodontium.

As pointed out before there are several clinical reports mentioning periodontal disease and alveolar bone loss in aircraft pilots exposed to high noise levels. This relation, as stated by the authors, is strongly correlated with flight hours.[13,14]

We found in previous studies, of LFN exposed animals, morphologic alterations, distortion and loss of the normal architecture of the periodontium – namely erosion of the bone surface and signs of bone necrosis, disappearance of the cementum and surface erosions on the root and deficient anchorage of the ligament – related to exposure, and similar to periodontal disease.[15]

In this study, we report an increase in collagen I with exposure. This is an expected result, as all the previous studies that have

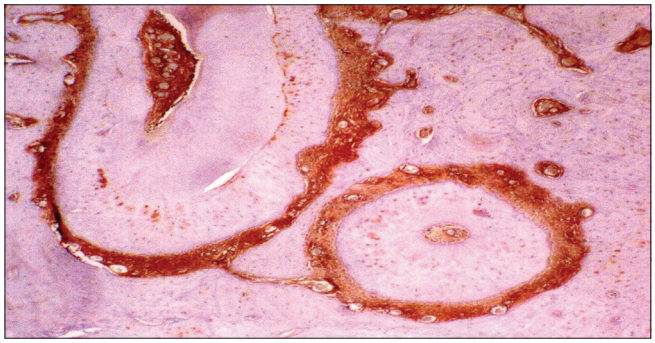

Figure 4: Periodontium imunolabeling for collagen IV (collagen I, ×200) gC

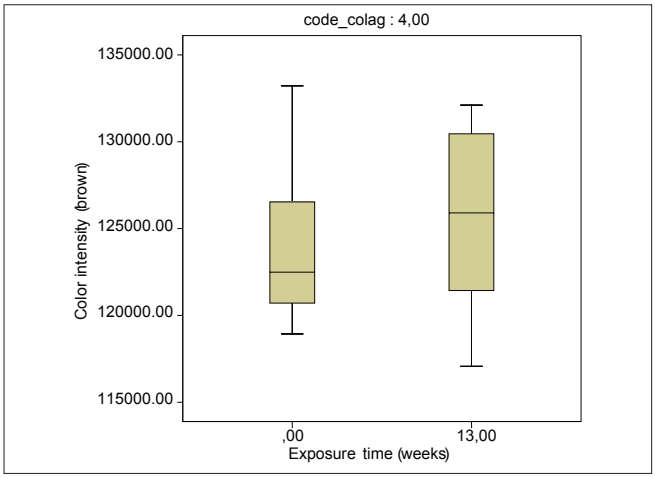

Figure 6: Immunolabeling for collagen type IV. There are no significant differences between groups

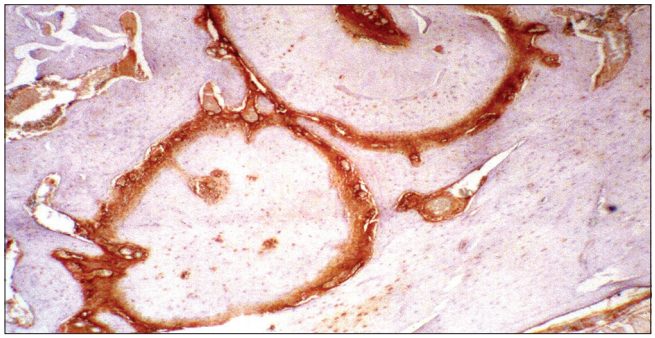

Figure 8: Periodontium imunolabeling for collagen V (collagen I, ×200) g13. the staining for collagen V increases with exposure to 13 weeks of low frequency noise

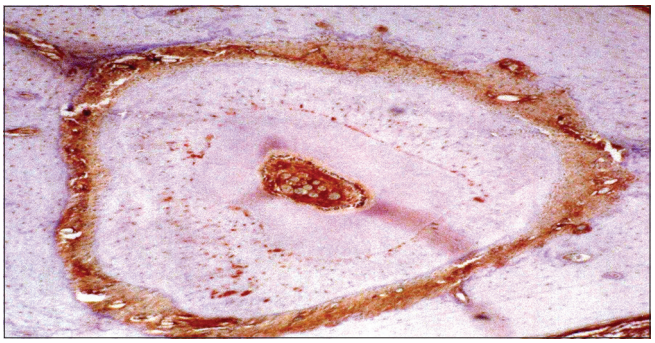

Figure 5: Periodontium imunolabeling for collagen IV (collagen I, ×200). g13. There is no increase in labeling for collagen IV in low frequency noise group

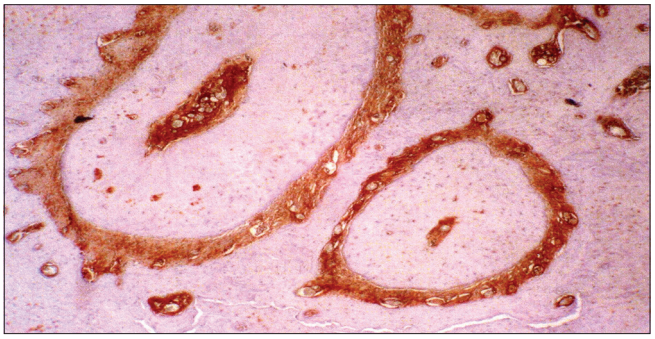

Figure 7: Periodontium imunolabeling for collagen V (collagen I, ×200) gC

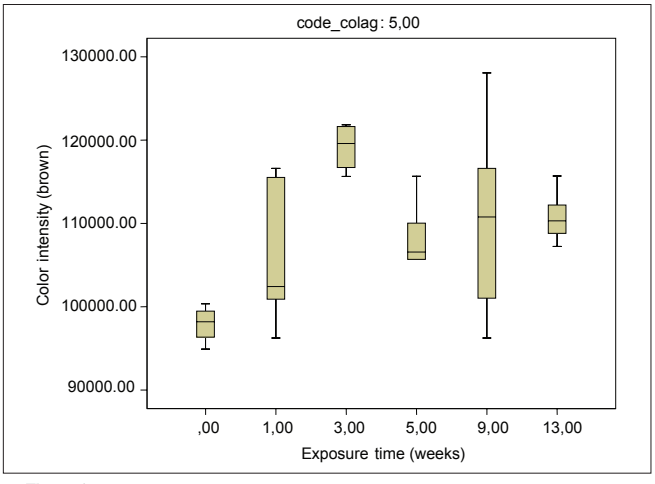

Figure 9: Immunolabeling for collagen type V. There are significant differences between gC and the group exposed to 13 weeks of low frequency noise

been done concerning non auditory health effects of noise describe proliferation of extracellular matrix and necessarily of collagen I, its main component. In the periodontium, the newly formed collagen is likely to be "non-functional" because beyond the loss of the typical architecture, collagen IV, that would be essential to blood vessel formation and to the vascular support of the newly formed tissue, remains unaltered. This fact leads us to the assumption that the increase in collagen I is similar to the fibrosis described by other authors in animals or humans.[2] Collagen V is necessary for the synthesis of collagen I and the increase of collagen I is normally accompanied by the increase of collagen V, so this result is a confirmation of the previous observations that we have made.

The results of this study are similar to those reported by Oliveira *et al*.[12] in the perivasculoductal connective tissue of the parotid gland, in which there is significant fibrosis and increase of collagen I and V in the connective tissue of the gland with exposure time, or to those reported by Fonseca *et al*., that described fibrosis in the stomach and duodenum walls and the same behavior in collagens.[10,16] Other organs such as vessels,[5,6] pericardium,[17] trachea,[18], or lungs,[19] have been shown to be affected by fibrosis when exposed. Ferreira *et al*.[18] stated that this proliferation of extracellular matrix could be a protective response against the direct mechanical aggression of sound pressure. A protective or even a recovery response from the periodontium exposed to LFN could explain the lack of differences between g3 and g13.

Clinically, altered collagen metabolism may predispose patients with diabetes to periodontal disease.[20] According to Lorencini *et al*.[21] the reorganization of extracellular matrix, namely collagen I and the development of the process of fibrosis should be considered in the progression of periodontal disease, and the fibrotic aspect caused by increased numbers of fibers and decreased number of cells, as found in our study, could create conditions for the appearance of periodontitis.[22]

The process of fibrosis and proliferation of extracellular matrix elements, including collagen I, is poorly understood in its cellular mechanisms handsets. It is known that mechanical stress can act as a stimulus for cell differentiation[23] and we believe, concerning fibrosis that the sound pressure could act as a mechanical stressor, inducing a response directly on the cells, by mechanotransduction.

The masticatory organ, the periodontium and the mouth have particular conditions that cannot be left apart when discussing these results. The possibility that noise might increase the masticatory strength causing stress to the periodontium, the reduction of saliva documented by Oliveira *et al*.,[11] together with the fibrosis can create conditions for the development of periodontal pathology. The results of the work we have been developing are consistent with clinical observations made by authors like Haskell and Carlson and Zackrisson.[13,14] Hence, we believe that a good environmental characterization is necessary in clinical studies concerning the periodontium and periodontal disease and effort needed to understand the cellular mechanisms altered by exposure to this kind of noise.

CONCLUSIONS

As in other organs, the newly formed fibrotic collagen is likely to be "non-functional," which could have clinical impact. These structural and functional alterations in the periodontium could contribute to the onset or the progression of periodontal disease.

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