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Original Article

Effects of industrial noise on circumpulpar dentin - a field emission scanning electron microscopy and energy dispersive spectroscopy analysis

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Abstract: Chronic exposure to Industrial Noise (IN), rich in Low Frequency Noise (LFN), causes systemic fibrotic transformation and sustained stress. Dental wear, significantly increased with exposure to LFN, affects the teeth particularly through the circumpulpar dentin. Our goal is to understand the consequences of IN exposure on the circumpulpar dentin of Wistar rats. 10 Wistar rats were exposed to IN for 4 months, according to an occupationally simulated time schedule and 10 animals were used as age-matched controls. The first and the second upper and lower molars of each animal were processed for observation by Field Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive Spectroscopy (EDS) analysis was performed. In exposed animals FESEM showed a 2.0 to 6.0 μm-dense mineral band between dentin and the pulp with no regular continuity with the tubules. This structure had a few tubules where the odontoblasts processes could be observed embedded within the band and collagen fibers were trapped inside. EDS analysis revealed that it was hydroxyapatite similar to dentin, with a higher carbon content. FESEM results show that the band may be tertiary reparative dentin formed by odontoblast-like cells, but the increased amount of carbon (EDS) could mean that it is sclerotic dentin. IN should be acknowledge as a strong stimulus, able to cause an injury to odontoblasts and to the formation of reparative tertiary dentin, in a process that may accelerate the aging of the teeth, either by direct impact of acoustic pressure pulsations or by increased stress and dental wear.

Keywords: Industrial noise, low-frequency noise, teeth, circumpulpar dentin, FESEM, EDS

Introduction

Noise exposure is an important health issue because certain professions, namely industry workers, are submitted to significant levels of wideband industrial noise (IN), rich in low-frequency components on a daily basis.

Low-frequency noise (LFN) has a deleterious effect on several organs and systems, mainly fibrosis, degenerative cellular changes and vascular lesions among others. In the oral cavity, Mendes *et al.* [1], described periodontal lesions and Oliveira *et al.* [2, 3], reported morphological and also functional alterations in the parotid gland.

Oliveira *et al.* [4] stated that, fibrotic transformation is a systemic effect of chronic exposure of rodents and humans to IN rich in LFN. The same authors concluded that chronic exposure to textile industry noise triggers cytological changes in the adrenals that suggest the existence of a sustained stress response. Previous studies demonstrated increased stress behaviour in patients exposed to LFN [5-8].

Dental wear has many causes and is significantly increased with exposure to LFN in an unpublished study by our group. Dental wear affects enamel and dentin, when dentin is affected, the pulp-dentinal complex is induced to react, particularly through the circumpulpar

Figure 1. Longitudinal fracture of the tooth in order to expose circumpulpar dentin of the wall of the horn (circle) and the chamber roof (arrow).

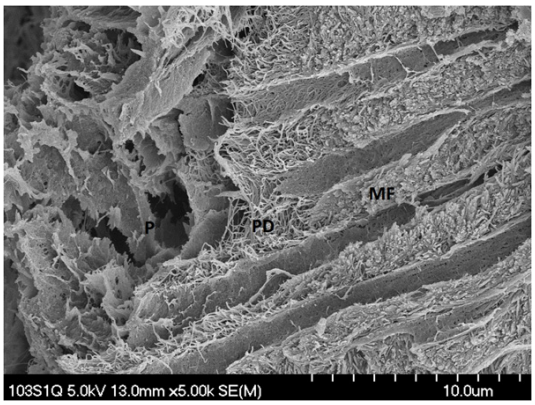

Figure 2. FESEM micrograph of the wall of the pulpar horn of an animal from the control group. P - pulp; PD - non-mineralized dentin (pre-dentin); MF - dentin mineralized front.

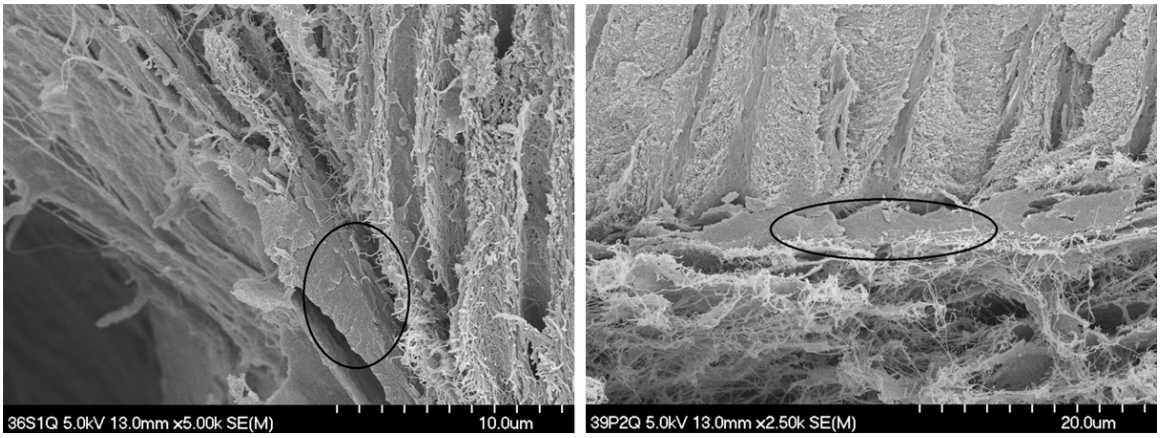

Figure 3. FESEM micrograph of the pulpar horn (left) and chamber roof (right) of an animal from the exposed group. Between the dentin and the pulp, a dense like-band can be observed (circle) with a width of 2.0 to 6.0 μm.

dentin. A significant degree of dental wear was documented by our group in rats exposed to LFN.

The goal of this study is to understand the consequences of IN exposure on the circumpulpar dentin of Wistar rats and to identify and characterize the alterations.

Materials and methods

Animals

We used 20 male Wistar rats, from a Spanish producer (Charles River Laboratories España SA, Spain). All animals were fed with standard rat food and had free access to water. They were kept under normal conditions and placed in groups of two inside a plastic box (42x27x16 cm) with a steel cover. The animals were treated according to the laws of the European Union and the Portuguese laws for experimental studies.

Noise exposure

The animals were exposed to industrial noise according to the protocol used by Oliveira *et al.* [9, 10].

10 rats were submitted to industrial noise for 4 months, according to an occupationally simulated time schedule (8 h/day, 5 days/week, and weekends in silence). The remaining 10 animals were used as age-matched controls (no noise exposure) and sacrificed after 4 months.

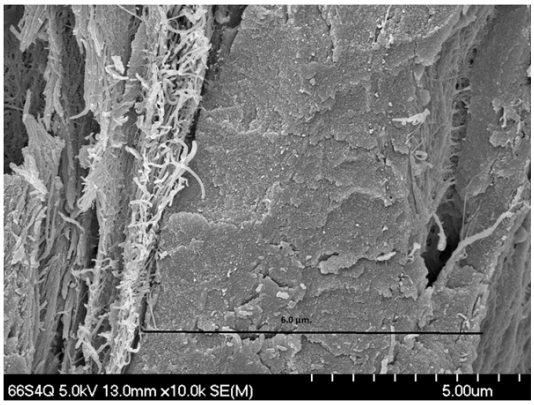

Figure 4. FESEM micrograph of the wall of the pulpar horn of an animal from the exposed group. This band-like structure has a width of 2.0 to 6.0 μm.

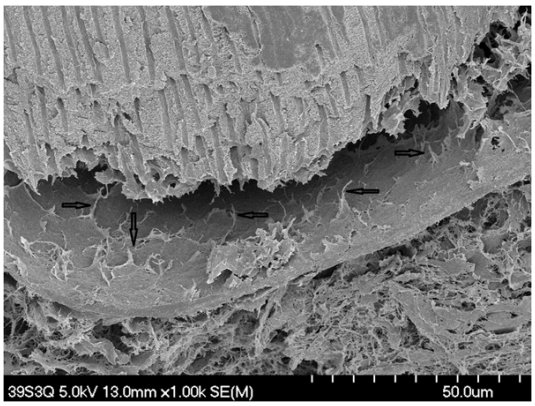

Figure 5. FESEM micrograph of the pulp chamber roof that is in contact with dentin. The mineral structure is separated from dentin. The almost compact structure has a few tubules where the odontoblastic processes (arrows) can be observed embedded within the mineral structure.

Figure 6. FESEM micrograph where the collagen fibers are surrounded by mineral (circle). Some collagen fibers are trapped within the mineral matrix (arrows).

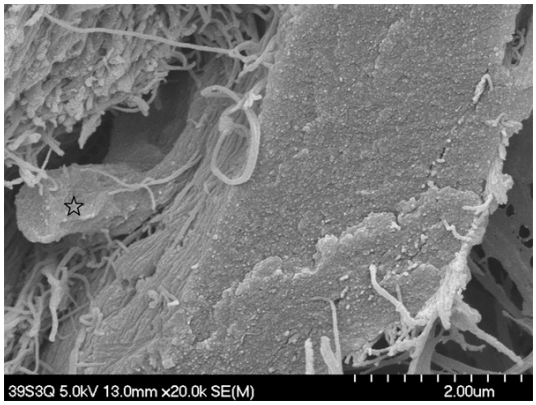

Figure 7. FESEM micrograph where it can be seen the projection-like "tag" (star) compatible with mineral. The fracture of the tooth shows that this mineral structure began its formation from the inside of the tubule.

The rats were sacrificed with a lethal intraperitoneal injection of sodium pentobarbital. We extracted the first and the second upper and lower molars of each animal.

The teeth were longitudinally fractured (in the mesio-distal direction) in order to expose the pulp chamber and channels and also the circumpulpar dentin (**Figure 1**). The specimens were immersed in a protease capable of digesting collagen, 2100 U collagenase, Clostridium histolyticum (Type II-S, C1764, Sigma-Aldrich, St. Louis, MO) in 0.05 M trometamol (TRIS) and 0.01 M for 1 month, with change of collagenase every 2 days. After deproteinization, we dehydrated the specimens according to the method used by Perdigão *et al*. [11]. The samples were then processed for observation by Field Emission Scanning Electron Microscopy (FESEM) under a microscope JEOL JSM-7001F and Energy Dispersive Spectroscopy (EDS) analysis were performed.

Results

FESEM observations showed a 2.0 to 6.0 μm-dense mineral band between dentin and the pulp, in the roof chamber and pulpar horn of all the animals exposed to IN (**Figures 3** and **4**). No such structure was found in the control animals (**Figure 2**). The mineralized structure did not have a regular continuity with the tubules. This almost compact structure had a few tubules where the odontoblasts processes

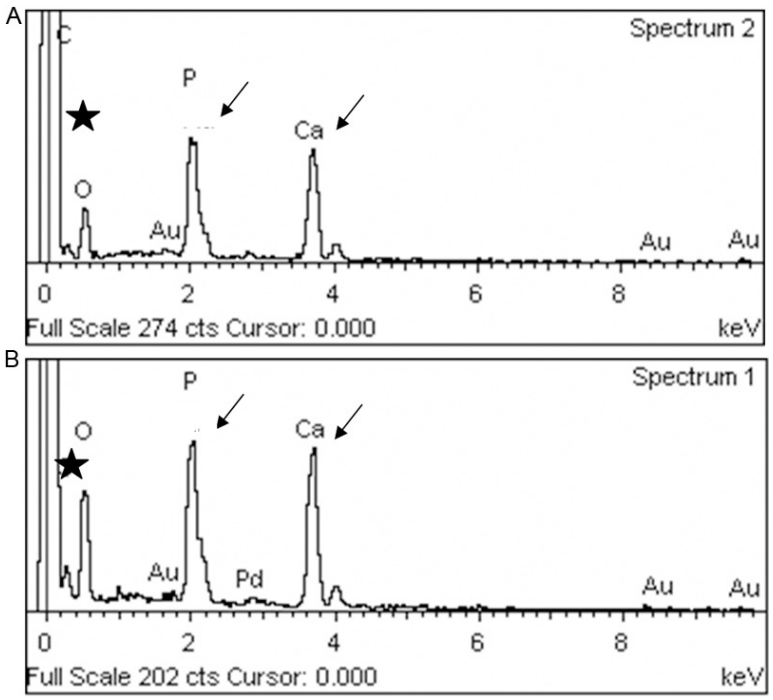

Figure 8. A: Typical dentin spectrum detected, with representation signal of calcium (Ca) and phosphate (P) (arrows). In biological apatite, the Ca/P mol ratio may range from 1.44 to 1.73. Carbon (C) and oxygen (O) was also detected (star). The gold peaks (Au) are due to the coating of the samples. B: Band-like structure spectrum detected, with representation signal of calcium (Ca) and phosphate (P) (arrows). Carbon (C) and oxygen (O) was also detected (star). Note that, even the absolute values of Ca and P are different on the band; the ratio of Ca/P is similar to the spectrum of dentin (arrows). C and O values indicate that this band is more carbonated than dentin (stars). The gold peaks (Au) and palladium (Pd) are due to the coating of the samples.

could be observed embedded within the mineral structure (**Figure 5**) and also collagen fibers trapped inside this mineral structure (**Figure 6**) and projection-like "tags" (**Figure 7**).

EDS analysis of this band revealed that it was hydroxyapatite and that the Ca/P ratio was similar to dentin, however carbon content showed higher absolute values in the band (**Figure 8A** and **8B**).

Discussion

Our results show the presence of a band-like structure between the pulp and dentin in the roof chamber of the teeth of IN exposed animals. These alterations may be related to the direct impact of sound pressure or to an adaptive response from the pulp-dentin complex related to teeth grinding.

Concerning the impact of LFN including infrasound, from industrial wind turbines and their impact in health, Ambrose and Rand [12] confirmed that these large wind turbines can produce real and adverse health impacts and suggest that this is due to acoustic pressure pulsations and not related to the audible frequency spectrum. Our group found evidence of LFN direct cellular lesion with functional repercussions in acinar salivary glands [2, 3], in the periodontium [1], in the myocardium [13, 14], in the stomach [15] and in the cilia lining of the pleura and of the trachea [9, 16].

Stimulation of odontoblasts induces an adaptive response characterized by altered functionality and synthesis of modified dentinal matrix [17-19]. According to Smith *et al.*, [17] the degree of each stimulus influences the odontoblast response differently. This remarkable adaptive capacity of the dynamic pulp-dentin complex is reflected in the formation of tertiary dentin. Compared with physiological dentin, the structure of tertiary dentin can be very variable in its morphology, its structure can contain fewer, irregular and constricted dentinal tubules [19].

Some studies [20, 21] distinguish two types of tertiary dentin: reactionary dentin being the tertiary dentin matrix secreted by surviving post-mitotic odontoblastic cells, in response to appropriate stimuli; and reparative dentin as a tertiary dentin matrix secreted by new odontoblast-like cells, after the death of the original odontoblasts, responsible for primary and secondary dentin secretion.

Under these assumptions reactionary dentin is formed responding to "moderate" stimuli and reparative dentin is formed responding to "strong" stimuli [17].

Stress and anxiety are "strong" stimuli related to teeth grinding. It is known that exposure to

LFN increases stress and our group has a study in which we showed that animals exposed to LFN have increased dental wear, so we cannot discard the possibility that the alterations we found are an adaptive response of the teeth to LFN induced stress.

EDS analysis, showed that the band-like mineral structure has a composition similar to hydroxyapatite. FESEM results show that the observed reparative form of tertiary dentin does not have tubular continuity with physiological secondary dentin matrix. So the band-like structure that we have found is probably tertiary reparative dentin, produced in response to the exposure to IN, by odontoblast-like cells.

Our spectroscopy results also revealed a Ca/P mol ratio of 1.448 in the normal dentin and a Ca/P mol ratio of 1.563 in the other circumpulpar mineralized structure, without significant differences. We cannot discard other types of dentin, like sclerotic dentin, that can have an increased mineral component [22]. According to Giachetti *et al.* dentinal sclerosis is a multifactorial event, due both to an increase in the thickness of peri-tubular dentin and to intra-tubular precipitation of calcium salts associated with mineralization of the organic structures present in the lumen. This type of dentin is more frequently found in older teeth [23]. In our study, we could not determine the mechanism that caused the mineral band formation. However, the morphologic aspect observed in our FESEM images shows that the mineral formation began from the inside of the tubules. Moreover, our results suggest that this mineralization occurred quickly, because we can observe the collagen fibers trapped inside the mineral structure. Although there are no significant differences in the mineral components, we have an atypical mineral formation and there is an increase in the amount of carbon in the mineral band, therefore we cannot exclude that the mineral band is sclerotic dentin. This could be justified by proximity to the pulp causing a sudden mineralization of the band. It is known that sclerotic dentin is related to the aging of the teeth [23, 24].

In conclusion, our results clearly show that IN should be acknowledge as a very strong stimulus, able to cause an injury to odontoblasts and, to the formation of reparative tertiary dentin, in a process that may accelerate the aging of the teeth, either by direct impact of acoustic pressure pulsations or by increased stress and dental wear.

Disclosure of conflict of interest

None.

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