ORIGINAL RESEARCH

Scaling and Root Planning decreases the Number of Melanosomes within Keratinocytes in Human Gingiva: Ultrastructural Analysis of Three Cases

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ABSTRACT

Aim: The aim of the present report was to evaluate the number of melanosomes within keratinocytes on pigmented gingiva, after and before scaling and root planning.

Materials and methods: Inflamed gingiva biopsies were taken from three patients (group 1). Forty days after scaling and root planning, biopsies were collected from the homologous contralateral areas (group 2). Samples were fixed in 2% glutaraldehyde—2.5% formaldehyde (freshly prepared from paraformaldehyde) in 0.1 M sodium cacodylate buffer, pH 7.4 for 4 hours, and then processed for transmission electron microscopy. Eighty electron micrographs were evaluated for recording the number of granules by a cross-section grid. The granules that were on intersections were recorded as well as the points that appeared on the cytoplasm for calculating the volumetric density (Vd), i.e the volume that the melanosomes occupied into the cytoplasm of keratinocytes. The presence of melanosomes in different stages of maturation and distribution into the cells were recorded with the aid of a magnifying glass. For the statistical analysis, a student t-test was applied.

Results: Results of the present report showed that melanosomes within keratinocytes were present in a higher number in inflamed gingiva A (11.08 ± 1.47), B (3.16 ± 0.38) and C (4.92 ± 0.89) and decreased after resolving of gingival inflammation A (9.46 ± 0.88), B (1.73 ± 0.25) and C (0.76 ± 0.18).

Conclusion: There is a possibility that inflammation influences the intensity of gingival melanin pigmentation.

Clinical significance: The periodontal treatment appears to have an effect on gingival melanin pigmentation.

Keywords: Gingivitis, Keratinocyte, Melanosome, Ultrastructure.

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INTRODUCTION

Melanin pigmentation on human epidermis and oral epithelium is related to the amount of melanin within keratinocytes, i.e. to the degree of maturation of melanosomes, to the number of melanosomes-containing keratinocytes, and to the pattern of distribution of melanosomes.[1] Thus, pigmented areas are clinically visible when the granules of melanin synthesized by melanocytes are transferred to keratinocytes.

Studies on distribution of melanosomes within keratinocytes have shown that the degree of pigmentation may be related to the number, size, composition and distribution of melanosomes, whereas melanocyte numbers typically remain relatively constant.[2-4] Thus, melanosomes present in epidermis or oral epithelium from blacks are larger than those from whites.[3]

Although the degree of melanin pigmentation in oral mucosa is mainly related to racial aspects, it was suggested earlier that tobacco smoking is associated with oral melanin pigmentation.[4] Indeed, some epidemiological

studies showed that 21 to 31% of smokers had clinically visible melanin pigmentation, whereas only 3% of non-smokers exhibited oral pigmentation.[5,6] In addition, smokers usually have more and deeper periodontal pockets as well as a higher mean probing pocket depth.[7,8] Therefore, it has been assumed that smoking may increase the severity of periodontal disease by potentially favor the increase of gingival inflammation.[9,10]

Although many investigators have draw attention at the different distribution patterns of melanosomes within keratinocytes from the different pigmentary races, the possible relationship between the presence of pigmentation and inflammation in gingiva was not well studied, except for the relationship smoking-inflammation-pigmentation mentioned above. By the other side, the number of melanosomes of the gingival epithelium per unit area is directly correlated with the severity of inflammation in the subjacent connective tissue of the attached gingival.[11] Therefore, the aim of the present report was to ultrastructurally evaluate the possible decreasing of melanosomes within keratinocytes on higher pigmented gingiva, after scaling and root planning, i.e. after reduction of inflammation in three patients by applying morphometrical analysis by transmission electron microscopy.

MATERIALS AND METHODS

Three white patients (mean age of 42.75 years) with gingivitis, in good physical health and free of medical complications, which had spots or band-like areas of melanin pigmentation on the attached gingiva were studied. All patients were fully informed about the procedures, and their written consent was obtained. This study was authorized by the Ethical Committee of the University of São Paulo, Brazil. Gingival condition was assessed according to the gingival index.[12] The Dummet-Gupta oral pigmentation index (DOPI) was used to evaluate the gingival pigmentation.[13]

Two *vs* three millimeters biopsies of inflamed gingiva (Fig. 1A) including marginal and attached gingiva (group 1) were taken from the buccal side, using the gingivectomy technique. The obtained samples were placed into a fixative solution, and then processed for transmission electron microscopy as indicated below. Then, the patients were submitted to oral hygiene instructions, scaling and root planning. When the gingiva exhibited clinical signs of health, i.e. without gingival inflammation, gingival bleeding (Fig. 1B), approximately after 40 days, new biopsies were collected from the homologous teeth and also processed for electron microscopy (group 2).

Biopsies were subdivided into approximately 1 mm thick slices cut vertically to the epithelial surface and following the imaginary long axis of tooth. Samples were fixed in 2% glutaraldehyde—2.5% formaldehyde (freshly prepared from paraformaldehyde) in 0.1 M sodium cacodylate buffer, pH 7.4* for 4 hours at room temperature. After washing for 1 hour in the same buffer, they were post-fixed in 'cacodylate-buffered 1% osmium tetroxide' (Sigma Chemical Co, St Louis, MO, USA) for 2 hours. All specimens were dehydrated in graded ethanols and acetone, and then they were embedded in Spurr resin.[†] Toluidine blue stained 1 μm thick sections were examined with a light microscope and regions containing the basal stratum of gingival epithelium were trimmed for ultrathin sectioning. Eighty nm thick ultrathin sections were cut with a diamond knife on a Leica Ultracut E ultramicrotome, collected onto copper grids, and stained with lead citrate/uranyl acetate. Ultrathin sections were examined and photographed in a JEOL 1010 transmission electron microscope as follows: Basal and suprabasal strata were examined and 10 keratinocytes containing melanosomes from each ultrathin section were randomly photographed at the same magnification (4,000×)

Figs 1A and B: Gingival appearance before (A) and after (B) scaling and root planning

using 8.3 × 10.2 cm electron microscope films (Kodak, Tokyo, Japan). Thus, a total of 20 electron micrographs (10 from group I and 10 from group II) with a final magnification of 16,000× were obtained from each patient.

Melanosomes were classified into four stages, according to the criteria from Jimbow[14] et al, i.e according to their degree of maturation: (I) membrane-delineated vesicles containing a proteinaceous matrix, (II) oval organelles with numerous membrane filaments, with or without cross-linking, but with a distinctive periodicity, (III) less periodicity and melanin deposition and (IV) a dense uniform particle without any distinguishable internal structure (the melanin granules).

A test system consisting of a square frame enclosing 1518 points marked as end points of 759 test lines with 0.3125 μm of length was used to analyze the electron micrographs (Fig. 2). In applying this test system, the test area amounted was 152.6 μm^2. Test points were differentially counted in order to estimate the relative volume (Vd) in μm^3 of melanosomes and other cytoplasmic components of a keratinocyte that appeared in the center of the micrograph.[15]

The points were differentially counted to estimate the relative volume (Vd) of the following tissues components: (1) melanosomes within keratinocytes (Pg), (2) keratinocyte cytoplasm (Pc) and (3) total of points upon melanosomes and keratinocyte cytoplasm (Pt = Pg + Pc).

The distribution of melanosomes within keratinocytes was obtained through the score of melanosomes. In addition, the morphological analysis of 4° of the melanosome maturation was performed using a magnifying glass (4×). The samples were distributed and student t-test was applied for the statistical analysis.

RESULTS

Two patients presented a DOPI of 1, while the remaining one patient exhibited a DOPI of 2. The mean gingival index decreased from 1.3 at baseline to 0 after periodontal treatment.

The melanosome number decreased in all patients from the noninflamed group, but the comparison between both groups showed statistically significant differences (p = 0.07). However, there was a high variability of melanosomes within cells from each patient (Table 1). When the number of the four stages of maturation of melanosomes was analyzed, stages III and IV were more frequently detected within keratinocytes (Table 1). In addition, melanosomes exhibited a statistically significant higher volumetric density (Vd) in the group 1 when compared to the noninflamed group (group 2) (p = 0.05) (Table 2).

DISCUSSION

The results of the present report show that the number of
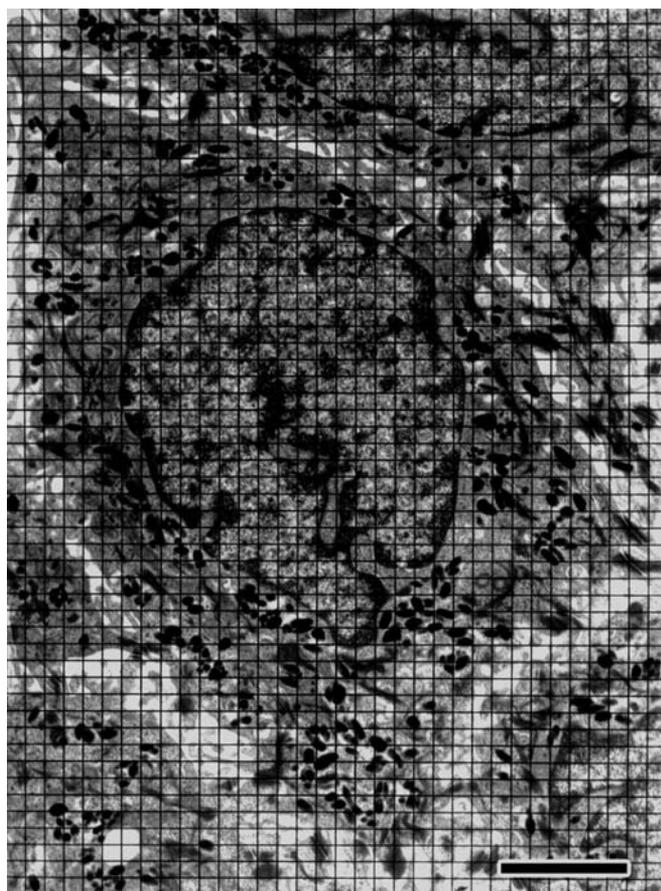

Fig. 2: One of the electron micrographs used in the present study in which a keratinocyte appears in the center of the micrograph. A test system consisting of a square frame enclosing 1518 points marked as end points of 759 test lines was superimposed to the micrograph. Each test line is 0.3125 μm in length (bar = 2 μm)

Table 1: Distribution of melanosomes according to their stage of maturation

	Baseline			Post-treatment		
	A1	*B1*	*C1*	*A2*	*B2*	*C2*
Stages of	*melanosome maturation*					
I	1	3	3	6	0	2
II	13	3	37	4	5	8
III	1228	234	612	340	130	42
IV	435	49	107	215	13	18
Total	1677	289	759	565	148	69

Table 2: Volumetric density (Vd) of melanosomes at baseline and post-treatment

	Patients		
	A	*B*	*C*
Baseline	11.0834[a]	3.1694	4.9255
	1.4703[b]	0.3871	0.8956
Post-treatment	9.4678	1.7351	0.7662
	0.8838	0.2540	0.1806

[a]Mean values of volumetric density in μm^3; [b]Standard error of mean values of volumetric density

melanosomes within keratinocytes in higher pigmented gingiva decreased after the removal of gingival inflammation. The effectiveness of scaling and root planning procedures on plaque and calculus removal that results in decreasing of gingival inflammation is one of the main principles of periodontal therapy. Elimination of supra and subgingival bacterial deposits reduces inflammation and ultimately arrests the progression of disease.[16-18] Besides reduction of inflammation after scaling and root planning was clearly observed clinically, our histological and morphometrical findings showed that the melanosome number inside oral keratinocytes actually decreased. This suggests that reduction of inflammation may influence on reduction of pigmentation. Although the number of melanosomes within keratinocytes decreased after surgical removal,[19,20] with reduction of pigmentation[21], the pigmentation the present findings suggest, for the first time, that reduction of gingival pigmentation may take place when inflammation is clinically resolved. This idea is supported by the observation of Lauand[22] et al (1981) in nonspecific and pregnancy induced gingivitis in which some morphological alterations were observed into melanoblasts, while a highly significant pigmentation was present in the inflamed gingival, and Patsakas[11] et al (1981) who observed that the density of melanosomes in the vestibular epithelium exhibited positive correlation with severity of inflammation in the attached gingiva.

The formation, maturation and trafficking of melanosomes is crucial to pigmentation, and defects in this process lead to depigmented and dilutionary disorders.[2] During the scaling and root planning invariably occurs the removal of part of the epithelium of gingival sulcus which can cause difficulty in the transport of melanosomes to queratinóciots, thus, affecting the pigmentation process.

CONCLUSION

In summary, despite the findings from the present report should be considered with caution due to the small sample size, they highlight to the possibility that some products derived from inflammation may have an effect on the behavior of melanocytes.

CLINICAL SIGNIFICANCE

The periodontal treatment appears to have an effect on gingival melanin pigmentation.

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