Gingival pigmentation due to prolonged retention of metal fragments

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Abstract : This report describes human gingival tissue reaction to prolonged retention of metal fragments which caused localized gingival pigmentation. Pigmented gingival tissues of the palatal gingiva and the mesial interdental papilla around the left maxillary first molar, which was restored with a cast core of silver alloy metal and a metal crown 6 years previously, were investigated with light, electron and electron probe microscopy. Even 6 years later, many black or dark brown deposits of various sizes were observed in the lamina propria mucosae of the patient’s gingival tissue. Electron-dense fine particles were observed within the cytoplasm of the fibroblasts. Macrophages containing the electron dense particles were occasionally observed. Examination by electron probe microscopy also revealed silver sulfate, tin sulfate, and iron in the deposits. Fine dust-like particles were also observed in the intercellular space of the gingival epithelium and the pinocytotic vesicles within the epithelial cells in which iron was also detected. Iron seemed to originate from a cutting steel instrument, since it is not a component of silver alloy. It appeared to be excluded by the cellular turnover. However, silver and tin were not detected in the epithelial cells or in the intercellular spaces. It was concluded that the gingival pigmentation caused by embedding of metal fragments would remain permanent, and not be diminished by cellular turnover.

Introduction

Dark gray or bluish purple gingival pigmentation around metal crowns is often found in clinical practice. A localized belt-shaped, patched or streaking pigmentation with a clear boundary is commonly observed at interdental papilla and marginal gingive at a short distance from the gingival crest. This kind of gingival pigmentation is seldom caused by systemic
Fig. 1 Pigmentation of the palatal gingiva of the left maxillary first molar (arrow) and mesial interdental papilla (arrowhead). Metal crown and cast core were removed.

Fig. 2 Light micrograph showing large (arrows) and small deposits (arrowheads) in the lamina propria just beneath the palatal gingival epithelium (GEP) of left maxillary first molar. ×740

disease or pathologic mucosal changes. One of the characteristic properties of this pigmentation is that it is generated near restored teeth. Since it is suspected that dental treatment procedures or restorative materials cause this kind of pigmentation, embedding of dental metal fragments such as amalgam and silver alloy metal in the soft tissue has been investigated by many researchers as the potential cause. However, there are few studies concerning human gingival tissue reaction to prolonged retention of dental metal fragments.

This study investigated human gingival tissue reaction related to the pigmentation caused by prolonged retention of dental metal fragments using light and electron microscopy, and electron probe microscopy.

Material and Methods

Pigmented gingival tissues of a 25-year old Japanese female were studied. Samples were taken from the palatal gingiva, and mesial interdental papilla around the left maxillary first molar, which was restored with a cast core of silver alloy and metal crown 6 years previously (Fig. 1). There was no pigmented gingiva around the abutment at that time.

The palatal gingiva was used for light microscopy and the mesial interdental papilla was used for electron microscopy. Tissues taken from both parts were minced into 1 mm segments. Then the segments were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 2 hours at room temperature. They were rinsed 3 times in the same buffer, stored in a fresh buffer overnight, then postfixed in 1% osmium tetroxide for 2 hours at 4°C. Samples were dehydrated in ascending concentrations of ethanol and absolute acetone, and embedded in Epon 812. The 1 μm thick sections were stained with toluidine blue for light microscopy. The thin sections were stained with uranyl acetate and lead citrate and then examined with a JEOL 1200 EX transmission electron microscope at 80 kv. The 0.5 μm thick and unstained sections mounted on nickel or copper grids were used for energy dispersive X-ray microanalysis using a Hitachi H-700 H analytical electron microscopy with a Si solid state detector of Kevex 7000 Q system.
Results

1. Light microscopical findings
Black and dark brown deposits of various sizes were observed in the lamina propria mucosae just beneath the gingival epithelium. Several large deposits (10-20 μm) seemed to be metal fragments in terms of form and size. Around these large deposits, a number of small deposits were also present (Fig. 2).

2. Electron microscopical findings
Multinuclear giant cells with a number of small deposits throughout the cytoplasm surrounded the large deposit. Both deposits were extremely electron-dense. Small deposits consisted of electron-dense fine particles. There were also many vacuoles varying in size (Fig. 3). Macrophage containing electron-dense particles were occasionally found. Fibroblasts were dispersed among the collagen fibers, containing numerous aggregations of electron-dense fine particles (10-30 nm in diameter). These aggregations were enclosed by a limiting membrane and were heterogeneous in shape, number and size. It was difficult to recognize lysosomes in fibroblasts containing many vesicles and endosomes (large vacuoles) filled with fine electron-dense particles (Fig. 4).

Fine dust-like particles were occasionally observed in the lamina propria mucosae just beneath the basal lamina and in the intercellular spaces of epithelial cells (Figs. 5 and 6). Numerous pinocytotic vesicles of the epithelial cells also contained these fine dust-like particles (Fig. 7). The gingival epithelium and lamina propria mucosae were intact and no inflammatory response was observed in the pigmented gingiva.

3. X-ray microanalytical findings
Point analysis of the aggregation of electron dense particles in fibroblasts shown in Figures 8 a, b. X-ray peaks of sulfur (Kα, Kβ), silver (Lα, Lβ), and tin (Lα, Lβ) were detected. Iron (Kα, Kβ) was detected in small amounts. The intensity of the X-ray peaks for...
Fig. 5 Electron micrograph of the fine dust-like particles (arrowheads) in the lamina propria mucosae. BL: basal lamina, C: collagen fibers, GEP: gingival epithelium. ×9,200

Fig. 6 Electron micrograph of gingival epithelial cells. Fine dust-like particles are present in the intercellular space (arrowheads). Dense granules in the epithelial cells are melanin granules (mg). BL: basal lamina ×6,000

Fig. 7 Electron micrograph of the pinocytotic vesicles (arrowheads) of the gingival epithelial cells (GEP), which contain fine dust-like particles. Fine dust-like particles (arrows) are present in the intercellular space (IS). ×33,000
Fig. 8  
(a) The points of analysis in the electron-dense fine particles in the fibroblast (arrow) and another fibroblast (cross point).
(b) X-ray spectra of the electron-dense fine particles in the fibroblast (arrow of Fig. 8 a) showing silver (AG) as the highest peak, tin (SN), sulphur (S), a little iron (FE) and copper (CU). X-ray spectra of the electron-dense deposition in another fibroblast (cross point of Fig. 8 a) are shown by the dotted line. The peak detected for copper appears to have been caused by the copper grid.

Fig. 9  
(a) The point of analysis (cross point) in the fine dust-like particles of the intercellular space of the gingival epithelial cell. Dense granules in the epithelial cells are melanin granules (arrowheads).
(b) X-ray spectra in the fine dust-like particles in the intercellular space showing a small iron peak (FE). Elements such as aluminium (AL), osmium (OS), chlorine (CL), and nickel (NI) seem to show background peaks caused by the fixative solution, resin and grids. S: sulphur, K: potassium.
sulfur, silver, tin, and iron varied in each cell. Elements such as aluminium (Kα), osmium (Mα, Lα), sulfur (Kα, Kβ), chlorine (Kα, Kβ), kalium (Kα, Kβ), iron (Kα, Kβ), and nickel (Kα) were detected in the fine dust-like particles within the intercellular space of the epithelium (Figs. 9 a, b).

**Discussion**

Experiments involving implantation of these alloys into the soft tissue of guinea-pigs and dogs revealed the mechanisms of pigmentation caused by dental alloys, rather than by dissolution from the crown margin3,6,7). However, little is known about the reaction of human gingival tissue to prolonged retention of dental alloy fragments.

In this case, even 6 years after cast core preparation, large and small deposits were still observed in the lamina propria mucosae beneath the gingival epithelium. The large deposit, assumed to be a dental alloy fragment, was surrounded by multinuclear giant cells. Macrophage and giant cells surrounding foreign bodies usually move to the lymph node3). In addition, some macrophages and multinuclear giant cells in amalgam tattoos have formed granulomas1-3). Formation of granulomas is considered a vital reaction in protecting the tissue from undigested foreign material in the absence of an inflammatory response8,9). In the present study, granulomas were not found. Although it is not entirely clear, in this case, that the multinuclear giant cells and macrophages containing the foreign material remained stationary for 6 years, new multinuclear giant cells and macrophages could have replaced the old cells during cellular renewal.

Within the cell bodies and the cytoplasmic processes of fibroblasts, a number of endosomes containing fine particles were observed. The cell organelles, such as rough endoplasmic reticulum, mitochondria, and Golgi apparatus were normal, and few lysosomes were observed despite the presence of many endosomes containing fine particles. The fibroblasts also appeared to function normally because of the collagen surrounding them. The question arises whether fibroblasts containing many endosomes filled with fine particles have cellular turnover. Based on the observation that a tattooed drawing hardly ever changes, Fujita et al.11) suggested that cellular turnover is very slow and during cellular renewal, the foreign material is transferred from the old fibroblast to the new cell.

The dental silver alloy consists mainly of silver and tin. In this study, silver and tin were probably released over time into tissue fluid by corrosion of the dental alloy fragments embedded in the soft tissue during dental procedures. These created sulfurated elements by combining with sulfur ions in the tissue fluid. This hypothesis is suggested by the X-ray peaks for silver, tin and sulfur in the deposits and the aggregation of fine particles.

The size and form of the fine dust-like particles observed in the epithelium and the lamina propria mucosae resembled those of the ferritin utilized to demonstrate the capacity for uptake, transport and retention of materials in human epidermis12). Ferritin entered the epidermis by crossing the basal lamina into the intercellular spaces and entered the cells via pinocytotic vesicles. Several researchers have also shown that substances such as particles and dyes can pass from the gingival connective tissue into the gingival sulcus13). These facts show that the gingival epithelium is capable of transporting materials. Fine dust-like particles, in which iron was detected, may have originated from a cutting tool, since iron is not a component of silver alloy. The iron diffusely penetrated the epithelium and entered the cell via pinocytotic vesicles. Finally, they appeared to be excluded by the cellular turnover. However, silver and tin were not detected in the epithelial cells or in the intercellular spaces. It seems that silver and tin deposits can not penetrate into the epithelium. These remain in the fibroblast permanently as metal tattoos. They can be removed only by surgical procedure.

**Conclusion**

This study demonstrated human gingival tissue reaction to prolonged retention of dental metal fragments. The following conclusions were drawn.

1. Even after 6 years, black and dark brown deposits of various sizes were present in the lamina propria mucosae just beneath the gingival epithelium.
and these may be related to dental alloy fragments embedded during the dental procedure.

2. Examination by electron-probe microscopy also revealed silver sulfate, tin sulfate and iron in the large deposits and small particles.

3. Fibroblasts contained many endosomes (large vacuoles) filled with electron-dense fine particles (10–30nm). Macrophages containing electron-dense fine particles were occasionally observed.

4. Fine dust-like particles found in the intercellular spaces of the gingival epithelium, and the pinocytotic vesicles within the epithelial cells, in which iron was detected, may have originated from a cutting tool. These may be excluded by cellular turnover.

5. Without surgical removal of the gingival tissue containing the fragments of the dental alloy and cells containing electron-dense fine particles, pigmentation of gingival tissue will remain permanently.

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References


