Archives of Oral Biology (2005) **50**, 89—96

Available online at www.sciencedirect.com

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Archives of Oral Biology

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SHORT COMMUNICATION

Ultrastructural study of calculus—enamel and calculus—root interfaces

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Accepted 1 July 2004

KEYWORDS
Calculus;
Calculus—tooth interface;
Ultrastructure;
Enamel apatite;
Cementum apatite;
Calcium phosphates

Summary

The attachment of dental calculus to the tooth (enamel or cementum) surface affects the ease or difficulty of its removal. Understanding the ultrastructural features of the calculus—tooth interface will help in the development of efficient strategies for efficient removal of dental calculus.
Objective: The aim of this study was to determine the ultrastructural characteristics of the calculus—tooth interface in relation to the occurrence of calculus fracture.
Design: Investigation of the ultrastructural characteristics of the calculus—tooth interface was made on eight human molars with mature supragingival and subgingival calculus using scanning electron microscopy (SEM), transmission electron microscopy (TEM) and fourier transform infra-red (FT-IR) spectroscopy.
Results: Fractures were shown by SEM to consistently occur within the calculus itself, but not at the calculus—tooth interface. Higher magnification revealed that the enamel apatite crystals (in the case of supragingival calculus) or the cementum apatite crystals (in the case of subgingival calculus) appeared intimately connected with the calculus crystals at the calculus—enamel or calculus—cementum interface. TEM micrographs confirmed this intimate direct connection or fusion (epitaxial growth) of calculus crystals with enamel and cementum apatite crystals. FT-IR showed lower concentrations of organic phase attributed to microorganisms and higher concentrations of collagen at the calculus—cementum interface compared to that in the calculus away from the interface.
Conclusion: Difficulty in complete calculus removal from tooth surfaces (especially from cementum or dentin) may be due in part to the intimate contact between the calculus and the tooth, due to the chemical bonding between the calculus crystals and the tooth apatite crystals and occasional fusion (i.e., epitaxial growth) of the

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doi:10.1016/j.archoralbio.2004.07.001

calculus calcium phosphate crystals with the enamel, dentin or apatite crystals. This cohesive bonding results in fracture planes occurring within the calculus instead of at the calculus–tooth interface.

Introduction

Dental calculus can form above (*supragingival calculus*) and/or below (*subgingival calculus*) the gingival margin. The mineral phase in enamel, dentin, cementum and bone consists of only one calcium phosphate phase, carbonate hydroxyapatite (CHA), except at the enamel surface which may also contain fluoridated hydroxyapatite.[1] In contrast, the mineral in human dental calculus consists of a mixture of calcium phosphate phases differing in crystal morphology, composition and dissolution characteristics.[2–8] The calcium phosphate phases include: CHA, dicalcium phosphate dihydrate (DCPD), octacalcium phosphate (OCP) and magnesium-substituted tricalcium phosphate (β-TCMP, whitlockite). Amorphous calcium phosphate (ACP) may also be present, but is difficult to detect by the usual analytical methods. The relative abundance of the different calcium phosphate phases depends on the age and location (supragingival, subgingival, labial or lingual) of the calculus.[2,7] The calcium composition (types and relative amounts of the different calcium phosphate phases present) can affect the chemical and mechanical properties of the dental calculus and therefore contribute to the ease or difficulty of its removal.[5,8] ACP, DCPD and OCP are more soluble than either β-TCMP or CHA.[4] It may be speculated that dental calculus consisting of more soluble phases and/or larger crystals may have lower attachment to the apatite crystals on the tooth surfaces (enamel, dentin or cementum) and therefore may make its removal easier.[5,8,9]

Although studies on ultrastructural features and composition of the calculus mineral phase have been reported,[2–8] only a small number of studies described the ultrastructural characteristics of the calculus–tooth surface interface.[9–11] Hayashi[10,11] investigated the initial stage of dental calculus formation on third molar human enamel using high resolution transmission electron microscopy (Hr-TEM) and electron diffraction. He reported that the calculus crystals consisted of needle-like hydroxyapatite crystals, with a direct connection between their lattice planes under Hr-TEM. He also demonstrated that lattice planes of calculus crystals directly coincided with those of enamel at the enamel–calculus junction. Although Hayashi provided valuable data on enamel–calculus interface, he examined only one sample at an early stage of calculus formation. Our study is the first ultrastructural characterization of the cementum–calculus interface.

The purpose of this study was to determine the ultrastructural features of the cementum–calculus and enamel–calculus interfaces in several teeth with mature calculus. Such information could provide insights into the significance of the calculus–tooth interface characteristics in relation to calculus fracture and calculus removal.

Materials and methods

Sample preparation

Eight human third molars (with subgingival and/or supragingival calculus) extracted for orthodontic purposes were used in this study. The thickness and colour of the calculus on these teeth compared to early calculus[8] suggested that these calculus specimens were mature (i.e., more than 3 months old). The teeth were dehydrated through different baths of ethanol (70, 80, 90, 95 and 100%, 2 h in each bath) and embedded in Epo-Fix resin. After polymerization (24 h at room temperature), the specimens were prepared by sectioning the calculus–tooth junction parallel to the long axis of teeth using Isomet. For the SEM analysis, the samples were coated with a thin layer of gold–palladium. For the TEM analysis, the areas containing calculus–tooth junctions (about 1 mm × 1 mm × 1 mm) were re-embedded in the Epo-Fix resin and sectioned (the same orientation as SEM specimens) with a thickness of 90 nm with a diamond knife using an ultramicrotome (LKB, Bromma, Sweden). The sections were mounted on Formvar-coated 100-mesh copper grids and then reinforced with a thin carbon coating. For the FT-IR analysis, the 3 μm sections (prepared using ultramicrotome) were mounted on BaF_2 discs.

Analytical methods

The ultrastructural characteristics of the interface between the calculus and the tooth (enamel and cementum) surfaces were analysed using a JEOL Transmission Electron Microscope, TEM, (CX-100) working at 100 kV; a JEOL Scanning Electron Microscope, SEM, (JSM-5400) working at 25 kV and a