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Chemical profile of the dentin substrate in non-carious cervical lesions

*Kunal Karan, Xiaomei Yao, Changqi Xu, Yong Wang**

University of Missouri-Kansas City School of Dentistry, Kansas City, 650 E. 25th St., Kansas City, MO 64108, USA

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ABSTRACT

Objective. The molecular structural nature of the dentin substrate in non-carious cervical lesions (NCCLs) is poorly understood. This investigation characterized the chemical structure including inhomogeneities, composition, mineral crystallinity, collagen organization of normal dentin and affected dentin substrates within NCCLs using Raman microspectroscopic mapping/imaging.

Materials and methods. Three extracted human pre-molars affected with NCCLs were selected and cavities matching the natural lesion with respect to size and location were prepared on the lingual/palatal surface of each tooth to serve as controls. The specimens were sectioned to expose the gingival and occlusal margins of the NCCLs and the control cavities. Micro-Raman spectra and imaging were acquired at 1.5 μm spatial resolution at positions perpendicular to the lesion surfaces.

Results. The Raman spectra and imaging comparisons showed the distinct compositional and structural alterations in mineral and matrix components of NCCL affected dentin. A heterogeneous hyper-mineralized layer, with characteristic features such as high phosphate/low carbonate content, high degree of crystallinity and partially denatured collagen were revealed in affected dentin substrate of NCCLs.

Significance. Generating Raman images based on different strategies from the same data set provides a powerful means to study the structural alterations within heterogeneous dental tissues. Direct overlay of the images indicated that the changes in chemical structure and composition are synchronized. Further studies are required to understand the role that these alterations play in response to acid etching and bonding to these clinically relevant substrates.

1. Introduction

Non-carious cervical lesion (NCCL) is a term used to refer to wasting diseases of dental hard tissue in the cemento-enamel junction region: this category includes abrasion, erosion and abfraction [1]. The NCCLs were first described by Zsigmondy in 1894 as angular defects [2]. In 1907, Miller defined abrasion, attrition and erosion as wasting disease of the teeth. More recently, Grippo added the term abfraction to this group of wasting diseases [3].

To achieve a desirable treatment outcome, it is important to have an understanding of disease mechanism and the changes it brings about in the dental hard tissue. Certain features like the presence of a 15-μm surface hypermineralized layer [4], physiological and pathological alteration in the dentin [5] and a marked reduction in the bond strength

* *Corresponding author*. Fax: +1 816 235 5524.
E-mail address: wangyo@umkc.edu (Y. Wang).
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of composite to these defects as compared to bonding to healthy dentin [6] have been reported. In total-etch systems, the failure of 40% phosphoric acid to preferentially dissolve intertubular dentin of NCCL-affected teeth and the inability of phosphoric acid to dissolve the more acid-resistant sclerotic mineral deposits present in the tubules may be responsible for the complete absence of resin tags in such dentin. The bond strength between the resin and the dentin substrate is also affected due to the alteration in the dentin substrate and a reduction in bond strength by up to 26% has been reported [7]. These contributory factors have a significant effect on the clinical performance of various restorative treatments as well as considerably lower 5-year retention rates for the resin composite restorations as compared to the resin modified glass ionomer restoration [8]. Resin composite systems rely only on micromechanical retention. Any alterations in the bonding substrate that interfere with such retention should certainly affect the longevity of these restorations in the oral cavity.

In view of these known facts, it is important to understand this clinically relevant substrate. A general consensus seems to be that current treatment protocols seem to be ineffective in creating desirable treatment outcomes. It is hoped that better knowledge of the structure and composition of this substrate can result in the creation of better durable bonds. Information about the morphological alterations in the dentin substrate in NCCLs is available, but a detailed ultra-structural chemical analysis of the substrate in these lesions is still lacking. This study was an attempt to answer some questions in this regard and to explore the alterations in the chemical structure and composition of the dentin in teeth affected by NCCLs using micro-Raman spectroscopy.

Raman spectroscopy is a technique used to study vibrational, rotational, and other low-frequency modes in material. It relies on inelastic scattering, or Raman scattering of monochromatic light, usually from a laser in the visible, near infrared, or near ultraviolet range. A Raman microscope begins with a standard optical microscope, and adds an excitation laser, a monochromator, and a sensitive detector (such as a charge-coupled device—CCD) or photomultiplier tube (PMT). Raman microscopy, and in particular confocal Raman microscopy, has very high spatial resolution. Since the objective lenses of microscopes focus the laser beam to several and/or sub-micrometers in diameter, the resulting photon flux is much higher than that achieved in conventional Raman setups. In the past, this technique had been used to a very limited extent to extract elaborate information about the chemical structure of mineralized tissue mainly due to the influence of fluorescence [9]. The confocal Raman microscope used in this study has the added benefit of enhanced fluorescence quenching.

In this study, the profile of changes in the chemical composition and structure of the NCCL affected dentin were studied as a function of the distance from the superficial layer toward the deeper layers of the substrate. The hypothesis tested was that as compared to normal dentin, NCCL affected dentin will exhibit a complex, non-uniform composition and structure as imaged in its natural, wet state using micro-Raman chemical imaging.

2. Materials and methods

2.1. *Specimen preparation*

Extracted human teeth with NCCLs (no = 3) were used for this study. The collected non-carious teeth were extracted for orthodontic or periodontal reasons other than the NCCL. Extracted teeth were collected from the Department of Oral Surgery, UMKC School of Dentistry in accordance with the IRB guidelines. The collected specimens were cleaned and then stored in 0.9% phosphate-buffered saline (containing 0.002% sodium azide) at 4 °C.

The specimens were then sectioned using a water cooled low speed diamond saw (Buehler Limited, Lake Bluff, IL, USA) and the occlusal crown and apical root was removed to isolate the coronal third of the root. After isolating the NCCL in the occlusal–apical direction, the outer 1/4th of the tooth structure was removed to generate sections 3.5–4 mm in width. This helped to expose the occlusal and gingival margins of the NCCLs. A specimen section used for this study is shown in Fig. 1. The prepared sections were cleaned under a jet of distilled water, then mounted horizontally in a petri dish using glue (Quick Gel, Henkel Loctite Corp. USA, Rocky Hill, CT, USA), covered with distilled water and set up for the micro-Raman (Olympus BX 41, Olympus America Inc., Center Valley, PA, USA) spectroscopic analysis.

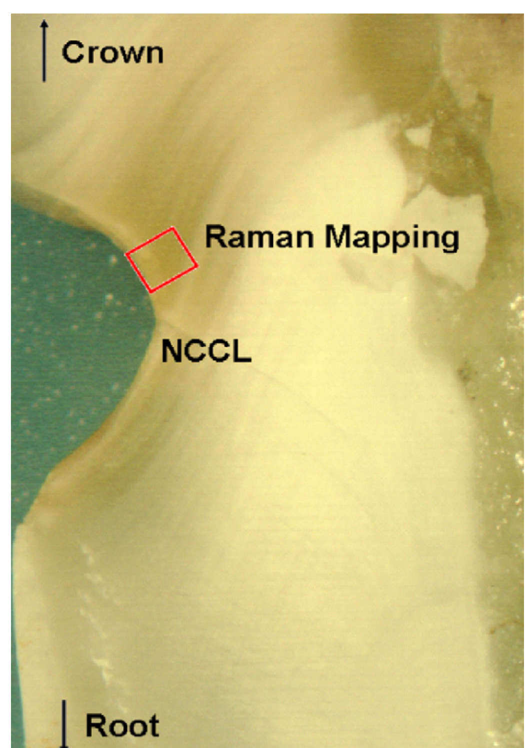

Fig. 1 – Light micrograph of a specimen section with NCCL. Also shown in the image is a representation of the area the micro-Raman mapping data were collected from.