Spatially Resolve Collagenase Resistance of Dentin with SR-FTIR

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Abstract

The bond between filling materials and the deeper portion of dentin is especially prone to be degraded in the long term. Dentin is composed mainly of hydroxyapatite and collagen matrix. Previous studies have related the bond instability to the abundance of collagenolytic enzymes in deep dentin, but the study regarding the collagenase resistance of dentin matrix is still lacking. Our study aims to spatially resolve the collagenase resistance by mapping the longitudinal sections of dentin after degradation. Specimens were first surface-demineralized with phosphoric acids based on the clinical procedures, then the specimens were challenged by immersing in the collagenase. After degradation, specimens were mapped with synchrotron-based FTIR (SR-FTIR) micro-spectroscopy and energy dispersive spectroscopy (EDS) to resolve the chemical components on the surface *in situ*. For FTIR spectra, amid I to phosphate absorbance ratio was utilized to quantify the collagen remnant on the surface. On the other hand, the normalized carbon percentage was presented to sketch the distribution of organic remnants. The results of SR-FTIR and EDS both indicated that the organic components gradually reduced along with depth after degradation, suggesting that the resistance to collagenase decreases along with the depth of dentin. By developing novel assays to map the organic remnants on the dentin surface, our study opens up new research opportunities and provides another theoretical basis of the depth-related bond instability.

Keywords – Dentin, Biostability, SR-FTIR.

Introduction

Dentin is a biocomposite composed mainly of the collagen matrix and hydroxyapatite. To achieve adequate adhesion to dentin, the substrate should be demineralized before filling. Nevertheless, it has been widely reported that the bond between filling materials and the deeper portion of dentin is especially prone to be degraded in the long term [1]. Even though a study has attributed the phenomenon to the presence of collagenolytic enzymes in deeper dentin [2], the collagenase resistance of the collagen matrix itself has never been discussed. As a result, the purpose of our study is to spatially resolve the collagenase resistance of dentin matrix with spectroscopic methods to further advance the longevity of tooth fillings.

Experiments

Sample preparation

Human teeth were longitudinally sectioned into 0.6-mm thick slices. The slices were surface-demineralized with 37% phosphoric acids according to clinical procedures to expose the collagen matrix. Afterward, the specimens were digested with collagenase from *Clostridium Histolyticum*. All samples were sequentially dehydrated with alcohol and vacuum dried for the subsequent analysis.

Synchrotron-based FTIR mapping

Specimens were characterized by acquiring the specular reflectance spectra with end-station of synchrotron-based FTIR (SR-FTIR) micro-spectroscopy in the BL14A1 at National Synchrotron Radiation Research Center (NSRRC). The spectra were acquired with accumulating 512 scans for every single point of 20×20 μm^2 on the sample surface irradiated by a focused infrared beam spot, confined using a confocal aperture. The field of view of a dentin sample section was 440×100 μm^2, mapped from outer to the inner border of dentin (Fig. 1). All specular reflectance spectra were corrected with the Kramers-Kronig transform for reducing the abnormal dispersion effect by using OMNIC™ 9.8 (Thermo-Fisher-Nicolet Scientific) and the baseline correction for further calculation.

SEM and EDS mapping

After coated with gold, samples were observed by Quanta 250 FEG (FEI) scanning electron microscope (SEM) in backscattered electron mode and mapped with QUANTAX EDS accessory (Bruker) using an accelerating voltage of 15 keV. Maps of respective elements, expressed by normalized atomic percentage, were output by ESPRIT software.

Data processing and Statistics

With SR-FTIR spectra, the matrix-to-mineral ratio was calculated as the absorbance ratio ($A_{1600\text{-}1700}/A_{1000\text{-}1080}$) of amide I to phosphates for the representation of the collagen remnant on the dentin surface [3, 4]. As for EDS mapping, the elemental profiles were acquired with ImageJ 1.53c. Statistics and data visualization were done with R x64 4.0.2 and Origin 8 software.

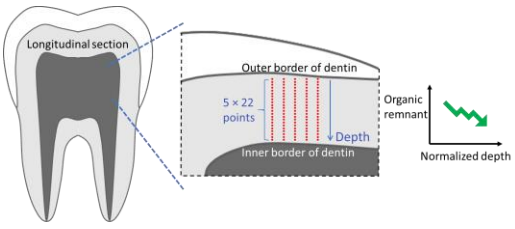

Fig. 1. Schematic diagram of the FTIR mapping set-up.

Results

The averaged spectra along with the depth of dentin exhibited that the absorbance of phosphates component increased with depth (Fig. 2). The trend was further illustrated by plotting the matrix-to-mineral ratio against normalized dentin depth (Fig. 3), suggesting the collagen matrix remnant on the surface decreased along with the depth. The results of FTIR spectra coincided with the carbon element profile in EDS analysis (Fig. 4), and the finding was further verified by SEM cross-sectional images (data not shown).

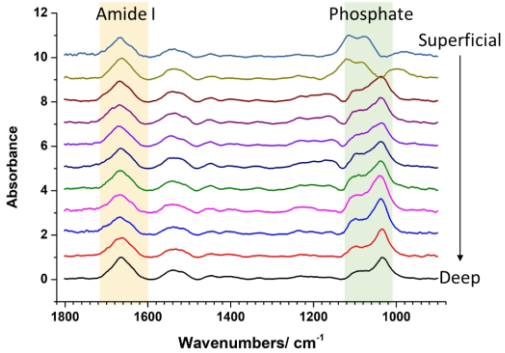

Fig. 2. 10-point averaged spectra from outer to inner aspects of dentin after degradation.

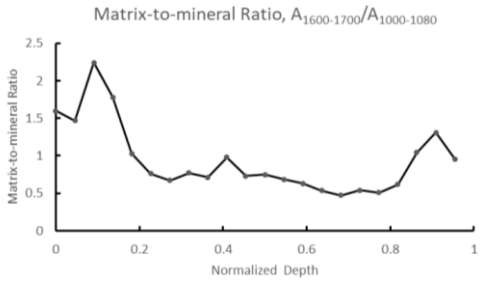

Fig. 3. Representative outer-to-inner matrix-to-mineral ratio profile according to the SR-FTIR spectra.

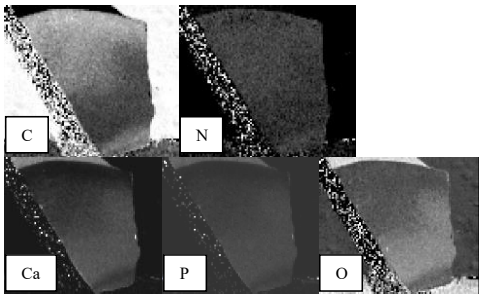

Fig. 4. EDS mapping of elements after degradation. Contrasts of individual elements were modified for better visualization of the trend.

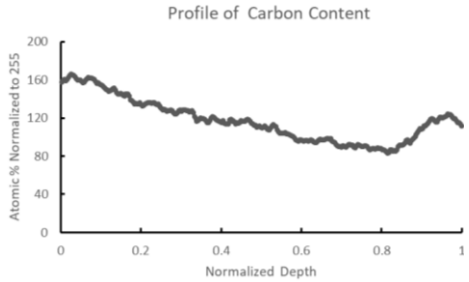

Fig. 5. Representative outer-to-inner profile of carbon element according to EDS analysis.

Discussion

Based on the results of EDS and FTIR mapping of dentin, the collagenase resistance of dentin decreased with the increasing depth. To our knowledge, the findings are reported for the first time. Our study provides another important basis for the depth dependency of bond degradation and is therefore worth further investigation.

Considering the non-contact and non-destructive nature of the reflective FTIR technique, we propose that FTIR micro-spectroscopy is a viable and novel tool to investigate dentin biostability *in situ*. Furthermore, FTIR micro-spectroscopy requires only minimal sample preparation, making it especially suitable for repeated measurements of biological samples. We are now exploring further applications of the powerful method along with EDS to resolve the cause of the phenomenon.

In conclusion, we successfully implemented two surface characterization techniques to resolve the collagenase resistance of dentin. The results indicated that deeper dentin is more likely to be degraded than superficial dentin. Accordingly, our study brings unique insights and can serve as a good platform for further research in the field of adhesive dentistry.

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References

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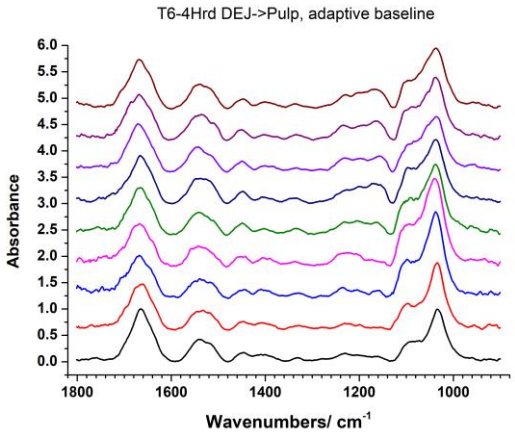

All presented data are from T6