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Photothermal radiometry and modulated luminescence examination of demineralized and remineralized dental lesions

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Abstract. Dental caries involves continuous challenges of acid-induced mineral loss and a counteracting process of mineral recovery. As an emerging non-destructive methodology, photothermal radiometry and modulated luminescence (PTR-LUM) has shown promise in measuring changes in tooth mineral content. Human molars (n=37) were subjected to demineralization in acid gel (pH 4.5, 10 days), followed by incubation in remineralisation solutions (pH 6.7, 4 weeks) without or with fluoride (1 or 1000 ppm). PTR-LUM frequency scans (1 Hz – 1 kHz) were performed prior to and during demineralization and remineralization treatments. Transverse Micro-Radiography (TMR) analysis followed at treatment conclusion. The non-fluoridated group exhibited opposite amplitude and phase trends to those of the highly fluoridated group: smaller phase lag and larger amplitude. These results point to a complex interplay between surface and subsurface processes during remineralization, confining the thermal-wave centroid toward the dominating layer.

1. Introduction

Dental caries is characterized by a dynamic equilibrium between cycles of acid-induced mineral loss (demineralization) originating in the teeth and mineral recovery from inorganic ions at the tooth-saliva interface to restore form and function (remineralization). The outer mineral component, enamel, contains about 87% volume mineral of impure carbonated hydroxyapatite (HAP), whose crystals are packed into prisms and oriented near-perpendicular to the tooth surface [1]. Weak acids present at the tooth surface create an acid gradient causing diffusion through enamel surface porosities resulting in a complex formation of multi-layered mineral structure of variable density over time and depth. The 3-layer geometrical profile of a demineralized lesion manifests itself as an intact surface layer of relatively high mineral volume superficial to a demineralized subsurface layer, where mineral volume may be as low as 50%, followed by intact enamel. The effect of fluoride on preventing and reversing early caries has been widely documented. Fluoride present in solution has been shown to enhance ingress of ions to deeper layers, promoting the precipitation of a less soluble mineral phase [2]. As a

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result, the mechanism of remineralization has been debated as being surface-limited or limited by diffusion of ions within the lesion and their subsequent precipitation reaction.

As laser based medical and dental systems are becoming more commonplace in clinical and laboratory environments, there is an increasing trend toward rapid evaluation of optical and thermal properties of biological tissues. In particular, coupled-field techniques have an advantage over conventional optical systems in resolution and the ability to employ comprehensive analysis of various parameters; such as optical and thermal properties in photothermal techniques [3]. Frequency-domain photothermal radiometry (PTR) detects the oscillatory temperature thermal-wave field induced by the optical-to-thermal energy conversion of a harmonically modulated laser beam. This creates diffuse-photon-density waves in turbid, optically and structurally anisotropic materials such as tooth enamel. Modulated luminescence (LUM) monitors the optical-to-radiative energy conversion, a complementary signal channel. The purpose of the present study was to use a remineralization system to relate changes in mineralization to changes in optical and thermal properties.

2. Methods

2.1 Sample preparation and treatment

Extracted, intact, human molars (n= 37) were sterilized by gamma irradiation (4080 Gy). Samples were submitted to demineralization (10 days) in 25mL of an acidified gel system (pH 4.5) [4] followed by remineralization (4 weeks; pH 6.7) in a calcifying solution [4] formulated without or with several fluoride levels. The prepared solutions were: 1) no fluoride solution (NF), 2) 1 ppm fluoride solution (LF) and 3) 1000 ppm fluoride solution (HF) (n=10/group). The remaining samples were demineralized only (n=7).

2.2 PTR-LUM experimental setup, scans and lesion validation

The PTR-LUM experimental setup has been detailed elsewhere [3, 4]. Experiments consisted of frequency scans measuring both amplitudes and phases of the PTR and LUM signals by varying the frequency from 1 Hz - 1 kHz. A frequency scan was performed before treatment on intact teeth and intermittently throughout both demineralization and remineralization treatments. At the conclusion of all treatments, samples were sectioned for transverse micro-radiography (TMR) followed by image analysis to produce quantitative parameters of integrated mineral loss (vol%.µm) and lesion depth (μm) [4].

3. Experimental results and discussion

Densitometric tracings from the demineralized samples produced caries lesions of poorer thermophysical properties than intact enamel, resulting in higher scatter of the diffuse-photon density field and confinement of the thermal wave centroid closer to the surface. This was accompanied by an increase in PTR amplitude with a concomitant decrease in PTR phase lag and a shift of the phase peaks to lower frequencies throughout demineralization (Figs. 1 and 2). LUM exhibited reduced amplitudes during demineralization, a result consistent with crystalline disintegration and enhanced lesion scattering. However, further monotonic shifts at the onset of remineralization may be attributed to the sensitivity of LUM to the state of hydration [4].

Individual remineralization treatments exhibited reductions in mineral loss and lesion depth compared to the demineralization group (students t-test, p<0.05). In the NF group (Fig. 1), the initial decrease in phase lag at high frequencies (f>100 Hz) at the onset of remineralization may be related to the enhanced mineral deposition in near-surface regions shifting the thermal wave centroid toward the exterior part of the lesion. This is further evident in the fact that low frequency amplitude and phase signals exhibit smaller changes compared to the response at high frequency. The increased amplitude and decreased phase lag with continued remineralization may be ascribed to a thickening upper layer, as evident in the TMR image, which may amplify the dominant surface reaction, thus concealing the effects of the subsurface layer as the thermal diffusion length becomes smaller compared to the



geometrical thickness. Furthermore, the shift of the phase peak maximum to slightly lower frequencies may be an artifact of a thickening surface layer.

Figure 1. Relative PTR-LUM amplitudes and phases normalized with respect to initial (intact) state for a sample in the NF treatment group. Images on the right display the densitometric tracing (top), calculated as an average across the TMR microradiographed lesion (bottom).

The onset of remineralization in the HF group (Fig. 2) induced an increase in phase lag at low frequency in conjunction with a significant reduction in PTR amplitude across the entire frequency range. This may be related to fluoride's known effect of enhancing the rate of mineral ion deposition throughout the depth of the lesion [5]. The increase in phase lag is associated with low-incident photon scattering and/or restoration of crystallinity and improved thermophysical properties, driving the thermal centroid deeper into the lesion resulting in a longer optical attenuation depth and larger phase lag of the photothermal response. This is evident in the lack of a visible lesion on the TMR image (Fig. 2). Fluoride-enhanced mineral deposition in the body of the lesion alters the 3-layer geometrical configuration into one of increasing subsurface homogeneity. Thus in the HF group, the increase in phase lag may indicate a regression in the underlayer, rather than restricting mineral deposition to the surface. However, the fact that PTR phase lag decreases in the high frequency range indicates the retreat of the thermal centroid in the opposite direction. This indicates a multi-factorial, complex, interplay during remineralization between the thickening surface layer, consistent with the smaller phase lag, and the regression of the demineralized underlayer, consistent with the larger phase lag. After a period of remineralization in the HF group, amplitude and phase signals conform to the NF group with decreasing phase lag and increasing amplitude, a fact that may be related to the dominating surface effects. This is supported by the fact that prolonged or high fluoride exposure induces mineral precipitation of a more stable fluoridated apatite phase on the enamel surface [2]. The result is visually white and opaque enamel, generating an optically highly scattering layer, which chemically amounts to an impedance to inorganic mineral ion diffusion into deeper layers, and physically creates a

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thermally thick layer of lower thermal diffusivity, apparent in the increasing amplitude and smaller phase lag.

Computational analysis to extract and characterize thermal and optical properties in these treated dental tissues can give insight into the mechanisms of de- and remineralization processes both quantitatively and non-destructively. Therefore, the next phase of this project involves multi-layer fits of intact, demineralized and remineralized curves in order to quantify and monitor changes in thermophysical parameters as a function of the treatment time. The implementation of the simplex downhill algorithm for the multi-parameter fits of the properties of intact teeth has been previously established [See Ref.3].



Figure 2. Relative PTR-LUM amplitudes and phases normalized with respect to initial state for a sample in the HF treatment group. Images on the right display the densitometric tracing (top), calculated as an average across the microradiographed lesion (bottom).

4. Conclusions

The present study further illustrates the multi-faceted and complex process of remineralization involving changes in multi-layer parameters towards a single homogeneous layer. This study advances the sensitivity of PTR-LUM to measure the effects of enamel remineralization, an advantage of its multiple signal channels and depth-profiling capabilities.

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