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Vertical scanning interferometry: A new method to quantify re-/de-mineralization dynamics of dental enamel

Isabella Pignatelli[a], *Aditya Kumar*[a], *Kumar Shah*[b], *Magdalena Balonis*[c,d,e], *Mathieu Bauchy*[f], *Benjamin Wu*[b,c,e,g,**], *Gaurav Sant*[a,g,*]

[a] *Laboratory for the Chemistry of Construction Materials (LC²), Department of Civil and Environmental Engineering, University of California, Los Angeles, CA 90095, USA*
[b] *Division of Advanced Prosthodontics, School of Dentistry, University of California Los Angeles, CA 90095, USA*
[c] *Department of Materials Science and Engineering, University of California, Los Angeles, CA, USA*
[d] *Institute for Technology Advancement (ITA), University of California, Los Angeles, CA, USA*
[e] *Department of Bioengineering, University of California, Los Angeles, CA 90095, USA*
[f] *Physics of Amorphous and Inorganic Solids Laboratory (PARISlab), Department of Civil and Environmental Engineering, University of California, Los Angeles, CA 90095, USA*
[g] *California Nanosystems Institute (CNSI), University of California, Los Angeles, CA 90095, USA*

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ABSTRACT

Objective. Remineralization and demineralization are processes that compete in the oral environment. At this time, numerous therapeutic agents are being developed to promote remineralization (precipitation) or suppress demineralization (dissolution). To evaluate the relative efficacy of such treatments, there is a need for non-invasive, real-time, high-resolution quantifications of topographical changes occurring during demineralization and remineralization.

Methods. Vertical scanning interferometry (VSI) is demonstrated to be a quantitative method to assess reactions, and topographical changes occurring on enamel surfaces following exposure to demineralizing, and remineralizing liquids.

Results. First, the dissolution rate of enamel was compared to that of synthetic hydroxyapatite (HAP) under acidic conditions (pH = 4). Second, VSI was used to compare the remineralization effects of F^--based and CCP-ACP agents. The former produced a remineralization rate of ≈349 nm/h, similar to simulated body fluid (SBF; concentration 4.6×) while the latter produced a remineralization rate of ≈55 nm/h, corresponding to 1.7× SBF. However, the precipitates formed by the CCP-ACP agent are found to demineralize 2.7× slower than that produced by its F^--counterpart.

Significance. Based on this new VSI-based data, a remineralization factor (RF) and demineralization (DF) factor benchmarked, respectively, to 1× SBF and the demineralization rate

* *Corresponding author at*: Laboratory for the Chemistry of Construction Materials (LC²), Department of Civil and Environmental Engineering, University of California, Los Angeles, CA 90095, USA.
** *Corresponding author at*: Division of Advanced Prosthodontics, School of Dentistry, University of California Los Angeles, CA 90095, USA.
E-mail addresses: benwu@ucla.edu (B. Wu), gsant@ucla.edu (G. Sant).
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of human enamel are suggested as *figures of merit* of therapeutic performance of dental treatments. Taken together, the outcomes offer new insights that can inform clinicians and researchers on the selection of remineralization strategies.

1. Introduction

Remineralization and demineralization are dynamic processes that compete in oral environments. While caries is associated with bacterial activity in dental plaque [1,2], erosion involves intrinsic and extrinsic acidic substances [3–9]. Both conditions result from imbalances that favor tissue demineralization. Demineralized hydroxyapatite (HAP) surfaces are more susceptible to other damage, e.g., abrasion, and the combination of acid dissolution and mechanical stresses promotes further enamel removal by exposing underlying tissues to successive acid attack [10–12]. As a result, there is significant interest in favoring remineralization and preventing demineralization. Besides enforcing dietary changes to reduce acidic food intake and hence demineralization [13], the routine use of topical agents can enhance remineralization [9,14–27].

Demineralization and/or remineralization of dental tissues has been widely studied using scanning electron microscopy [28], transmission electron microscopy [29], atomic force microscopy [30], indentation [31], microradiography [32], electron probe microanalysis [33], light induced fluorescence [34], secondary ion mass spectroscopy [7], confocal microscopy [35], electrochemical impedance spectroscopy [36], ultrasonic measurements [37], iodide permeability test [38] and contact profilometry [39]. However, microscopy techniques only offer qualitative 2D means for assessment [40]. Other techniques, e.g., microradiography and transmission electron microscopy are destructive and do not provide real-time evaluation. Further, the experimental conditions can also be limiting—e.g., in the case of microradiography the alignment and geometry of the X-ray beam can limit imaging precision at the sample edge [40]. While methods such as atomic force microscopy offer nanoscale resolution, the measurements are time-consuming [7] and of limited statistical relevance, as only small lateral areas can be scanned in realistic durations.

Recently, non-contact profilometry (e.g., laser scanning or white light interferometry, WLI) has been proposed as a new method to study dental tissues [10,14–17,41–45]. By building on past studies, this paper establishes procedural details for full-field quantitative assessments of reactions (i.e., dissolution or precipitation) occurring on dental tissue surfaces in contact with a solution using vertical scanning interferometry (VSI). VSI allows quantifications of surface topographies with a vertical resolution of ≈0.1 nm and a lateral resolution of around 500 nm. VSI uses interferometric objectives consisting of an objective lens, a reference mirror and a beam-splitter (see Fig. 1). The most common interferometric objectives differ in terms of the mirror location, such that the mirror can be located between the objective lens and the beam-splitter (Mirau objective) or it can be placed in another position because of its larger size (a Michelson objective). In both cases,

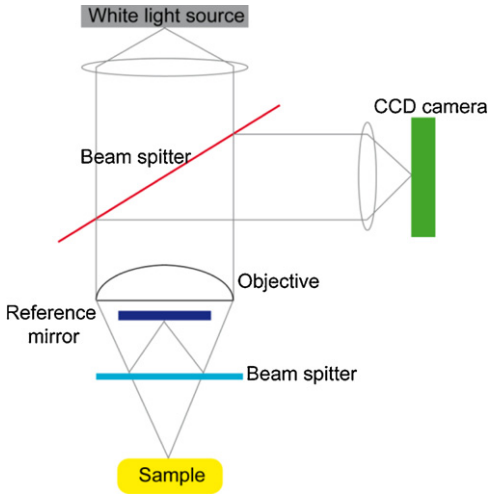

Fig. 1 – A schematic illustration of a vertical scanning interferometer (VSI) showing the different imaging components.

a source directs a light beam onto the sample surface through the interferometric objective, where the beam-splitter separates the light into two beams. One beam is reflected back by the reference mirror, while the other travels along the optical axis and interacts with the sample. This latter beam is reflected by the sample's surface. This results in an optical path difference between the two light beams and, a pattern of interference fringes forms when the beams are recombined.

This interference pattern is composed of light and dark bands: when the two beams are in phase their amplitudes are summed and a light band forms, whereas when the beams are out of phase their amplitudes are subtracted and a dark band of zero amplitude results. The interference fringes are sampled by a CCD (charge-coupled device) sensor and the signal is digitized and processed to obtain 3D topographical maps of the sample's surface. VSI offers distinct advantages over other techniques, including: (i) non-destructive evaluation, (ii) nanoscale resolution of surface profiles (≈0.1 nm in the z-direction) with the ability to scan large lateral areas on the order of 10 s of mm^2, in real time, and (iii) the ability to render statistically relevant quantifications of true reaction (dissolution or precipitation) rates, while duly accounting for the exposed (surface) area of the solid. Therefore, this paper uses VSI to quantify the dissolution rate of enamel and synthetic HAP at pH 4. Further, the remineralizing effects of fluoride and CPP–ACP toothpastes are measured with subsequent analysis of demineralization behavior of the remineralized enamel surfaces.

The first aim of this study is to establish VSI as a quantitative tool to evaluate demineralization and reminer-