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Technical note

Detection of dentinal cracks using contrast-enhanced micro-computed tomography

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ARTICLE INFO

Article history:

Received 12 June 2009

Received in revised form

17 September 2009

Accepted 1 October 2009

Published online 13 October 2009

ABSTRACT

A new technique using contrast enhanced micro-computed tomography (micro-CT) was developed to improve the ability to detect dentinal cracks in teeth and assess associated risks to oral health. Extracted, whole human molars that exhibited visual evidence of external cracks following extraction and machined, partially fractured elephant dentin specimens were labeled by BaSO₄ precipitation and imaged by micro-CT. Contrast-enhanced micro-CT was demonstrated *in vitro* to enable non-destructive, 3-D imaging of the presence, morphology and spatial location of dentinal cracks in whole human molars and machined specimens. BaSO₄ staining provided enhanced contrast for the detection of cracks that could not be detected prior to staining. Backscattered SEM micrographs showed that BaSO₄ was precipitated on the surfaces of dentinal cracks and within adjacent tubules. The new methods demonstrated in this study are expected to be useful for clinical and scientific studies investigating the etiology and treatment of dentinal cracks in teeth.

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1. Introduction

Cracked teeth are commonly observed in dental practice and are potentially symptomatic (Clark et al., 2003; Kahler, 2008; Snyder, 1976). Cracked teeth are the third most common cause of tooth loss after caries and periodontal disease (Braly and Maxwell, 1981; Hiatt, 1973). Treatment options are typically lengthy, expensive and include a risk of morbidity (Liu and Sidhu, 1995; Homewood, 1998;

Geurtsen and García-Godoy, 1999; Kahler, 2008), yet there is little definitive evidence to support the diagnosis of cracks requiring intervention or the effectiveness of interventions (e.g., Bader et al., 1996). Therefore, treatments may be either over-prescribed or prescribed too late.

Current methods for the diagnosis of cracks that may compromise teeth are all based on optical assessment, with or without the aid of surgical loupes, microscopes, dyes and/or transillumination (Clark et al., 2003; Kahler, 2008). Improvements in the detection and classification of cracks have been demonstrated using enhanced magnification

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doi:10.1016/j.jmbbm.2009.10.003

(Clark et al., 2003; Slaton et al., 2003) and transillumination (Wright et al., 2004). However, optical methods suffer from an inherent inability to assess the severity of cracks (e.g., crack depth), particularly sub-surface dentinal cracks. Moreover, dentinal cracks are generally not able to be detected in plain radiographs.

Micro-CT is useful for non-invasive, three-dimensional (3-D) imaging of the internal structure of mineralized tissues, due to relative differences in x-ray attenuation. Cone beam volumetric micro-CT was recently demonstrated to provide more accurate assessment of proximal caries lesion depth compared to conventional radiographs (Kalathingal et al., 2007). Intra-oral systems limit the volume irradiated and thus achieve a low radiation dose to the patient; however, the spatial resolution is currently limited to 40 μm and the image contrast is low relative to conventional CT (Kalathingal et al., 2007). High-resolution micro-CT systems are commercially available for both *in vivo* imaging of small animals and *in vitro* imaging of tissue specimens. The spatial resolution is typically on the order of 10 μm and systems with 1 μm resolution are now available. However, the detection of small cracks ($\sim 1 \mu\text{m}$ in width) in relatively large specimens (e.g., a molar) is limited by the resolution, maximum specimen size, and data collection time of the above instruments. X-ray tomography using high energy, monochromatic synchrotron radiation is able to image cracks in dentin (Kruzic et al., 2003), but is neither readily available nor amenable to imaging large numbers of tissue specimens that are relatively large in size.

Non-destructive, 3-D imaging of the presence, spatial location and accumulation of microdamage in bone tissue was recently demonstrated for the first time using contrast-enhanced micro-CT with a precipitated BaSO_4 stain (Leng et al., 2008; Wang et al., 2007). Although imaging of cracks and damage in bone had been previously demonstrated using high energy, monochromatic synchrotron radiation (Nalla et al., 2005), this approach enabled the use of commercially available bench top micro-CT instruments. BaSO_4 was precipitated within damaged tissue, cracks and vasculature, as verified by backscattered electron imaging and energy dispersive spectroscopy (Leng et al., 2008; Wang et al., 2007). Moreover, precipitated BaSO_4 enhanced the intensity of voxels in micro-CT due to the higher x-ray attenuation of BaSO_4 relative to bone tissue. The micro-CT scanner (10 μm resolution) was unable to detect microcracks or fatigue cracks without the use of the contrast agent. Therefore, contrast-enhanced micro-CT is expected to find increased use in the study of mineralized tissue mechanics, complementing or replacing methods of damage/crack detection that are inherently destructive, two-dimensional and tedious.

The objective of this study was to non-destructively and three-dimensionally image dentinal cracks using contrast-enhanced micro-CT. Extracted, whole human molars that exhibited visual evidence of external cracks following extraction and machined, partially fractured elephant dentin specimens were labeled by BaSO_4 precipitation and imaged by micro-CT using methods adapted from previous studies investigating microdamage in bone tissue (Leng et al., 2008; Wang et al., 2007).

2. Materials and methods

2.1. Elephant dentin

Two partially fractured, dehydrated compact-tension specimens of dentin from tusk shards of a single adult male elephant (*Loxodonta africana*) used in a previous study (Kruzic et al., 2003) provided well-characterized and highly controlled cracks for evaluating feasibility of the technique. Elephant dentin is similar to human dentin in containing tubules within an extracellular matrix of mineralized collagen fibers and avoids the specimen size limitations of human dentin. Compact-tension specimens were machined while hydrated to a thickness of $\sim 2.5 \text{ mm}$ and width of $\sim 15 \text{ mm}$. Specimens were polished to a 1200 grit finish, followed by 1 and 0.05 μm alumina suspensions. An initial notch was introduced with a low speed diamond wafer saw such that the crack growth direction was oriented perpendicular to the long axis of the tubules and the crack plane was in the plane of the tubules. A final notch with root radius $\sim 15 \mu\text{m}$ was achieved by repeatedly sliding a razor blade over the initial notch. Fracture resistance (R-curve) testing was conducted *in vacuo* ($\sim 10^{-4} \text{ Pa}$) in a scanning electron microscope (SEM). Specimens were rehydrated and stored in phosphate buffered saline upon receipt and during all interim periods before staining and micro-CT.

2.2. Whole human molars

Ten extracted human molars from different donors were provided by the Practice-based Research in Oral Health (PROH) practitioner network through the Oregon Health and Science University. Teeth were screened after extraction for evidence of external cracks upon inspection with 3.5X surgical loupes. All teeth were maintained hydrated in a 0.05% solution of chloramine T during all interim periods before staining and micro-CT.

2.3. Histological staining and imaging

Elephant dentin specimens and whole human molars were imaged before and after histological staining by micro-CT ($\mu\text{CT-80}$, Scanco Medical AG, Bassersdorf, Switzerland) at 10 μm resolution, 70 kVp voltage, 113 μA current, and 400 ms integration time. Grayscale images were smoothed by Gaussian filtering. High intensity voxels representative of BaSO_4 were segmented to highlight cracks in 3-D reconstructions. An image subregion was used to remove the effect of non-specific BaSO_4 staining on specimen free surfaces which would have otherwise obscured the visualization of internal features in 3-D reconstructions.

All specimens were stained by BaSO_4 precipitation, soaking in an equal parts mixture of 0.5 M barium chloride (Certified ACS crystal, Fisher Scientific, Fair Lawn, NJ) in de-ionized water, buffered saline, and acetone for 3 days, followed by an equal parts mixture of 0.5 M sodium sulfate (Anhydrous powder, Fisher Scientific, Fair Lawn, NJ) in de-ionized water, buffered saline, and acetone for 3 days, both under vacuum ($\sim 50 \text{ mm Hg}$). The staining mechanism was a precipitation reaction where $\text{BaCl}_2(\text{aq}) + \text{Na}_2\text{SO}_4(\text{aq}) \rightarrow \text{BaSO}_4(\text{s}) + 2\text{NaCl}(\text{aq})$. Barium and sulfate ions diffused into

and concentrated within void space in the dentinal tissue – e.g., tubules and cracks – which acted as a “micro-reactor” and provided an abundance of heterogeneous nucleation sites on tissue surfaces (Leng et al., 2004, 2008).

After staining and micro-CT, specimens were dehydrated in a graded series of alcohol solutions, dried overnight in an oven at 90 °C, embedded in poly(methyl methacrylate), sectioned with a low-speed diamond wafer saw, polished with a series of diamond compounds to a 1 μm finish, and coated with Au–Pd by sputter deposition for scanning electron microscopy (SEM). Specimens were imaged using backscattered electrons (Evo 50, LEO Electron Microscopy Ltd., Cambridge, UK) at an accelerating voltage of 20 kV and a working distance of 9–12 mm. Note that image contrast from backscattered electrons is primarily due to compositional differences in atomic number, with an increasing atomic number resulting in increased intensity. The elemental composition of the stain was verified by electron probe microanalysis using energy dispersive spectroscopy (EDS) (INCA x-sight model 7636, Oxford Instruments America, Concord, MA).

3. Results

Contrast-enhanced micro-CT enabled non-destructive, 3-D imaging of the presence, morphology and spatial location of dentinal cracks in machined specimens (Fig. 1) and whole human molars (Fig. 2). In both types of specimens, BaSO₄ staining provided enhanced contrast for the detection of cracks that were not able to be detected prior to staining (Figs. 1(b), (c), and 2(b)). Note that the entire 3-D volume of grayscale image slices for the specimens shown in Figs. 1 and 2 are available as movie files (.avi) in Electronic Annex 1 and 2, respectively, of the online version of this article. Backscattered SEM micrographs showed that BaSO₄ was precipitated on the surfaces of dentinal cracks and within adjacent tubules (Fig. 1(e)). The highest levels of image intensity for both micro-CT and backscattered SEM were shown to correspond to the presence of elemental barium and sulfur measured using EDS. At higher magnification, SEM revealed the presence of BaSO₄ crystals and aggregates, typically submicron but up to 5 μm in size, on the surfaces of propagating cracks and within adjacent tubules. BaSO₄ was also precipitated on all free surfaces, including the root canal and pulp chamber (Fig. 2(a)). Therefore, a subregion was used to remove staining of the outer specimen surfaces and reveal internal features, including cracks, in segmented 3-D reconstructions (Figs. 1(a), and 2(a)).

4. Discussion

The feasibility of non-destructive, 3-D imaging of dentinal cracks was demonstrated *in vitro* in machined, partially fractured specimens (Fig. 1) and whole human molars (Fig. 2) using contrast-enhanced micro-CT with a precipitated BaSO₄ stain. BaSO₄ precipitation on the surfaces of dentinal cracks and within adjacent tubules enhanced the intensity of voxels in micro-CT due to the higher x-ray attenuation of BaSO₄

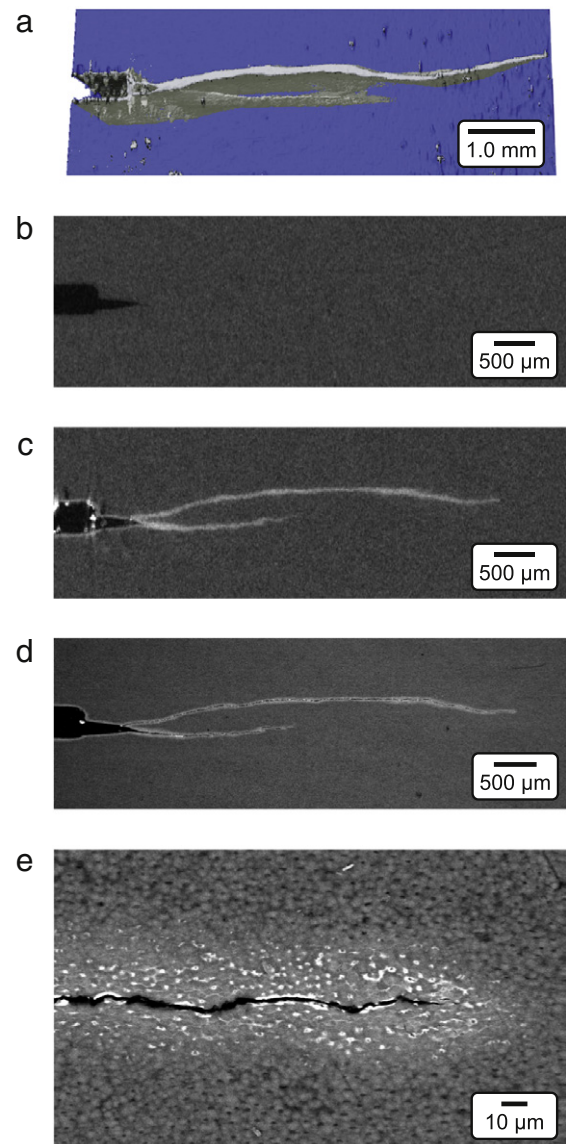


Fig. 1 – A branched crack that propagated from a notch (left) in a compact tension specimen of elephant dentin was imaged by micro-CT before and after staining by BaSO₄ precipitation, and by backscattered SEM after staining by BaSO₄ precipitation. (a) A segmented, three-dimensional micro-CT reconstruction showed crack surfaces stained with BaSO₄. Cross-sectional grayscale micro-CT images at the same depth approximately midway through the specimen thickness (b) before and (c) after staining by BaSO₄ precipitation showed that the crack was unable to be detected at this location without the use of the contrast agent. The contrast-enhanced micro-CT image in (c) was compared to (d) a backscattered SEM micrograph for the same specimen at approximately the same cross-sectional depth and magnification. (e) A backscattered SEM micrograph of the crack tip in (d) at higher magnification showed BaSO₄ penetration into microtubules. Note that the presence of elemental Ba and S was verified by EDS (not shown).

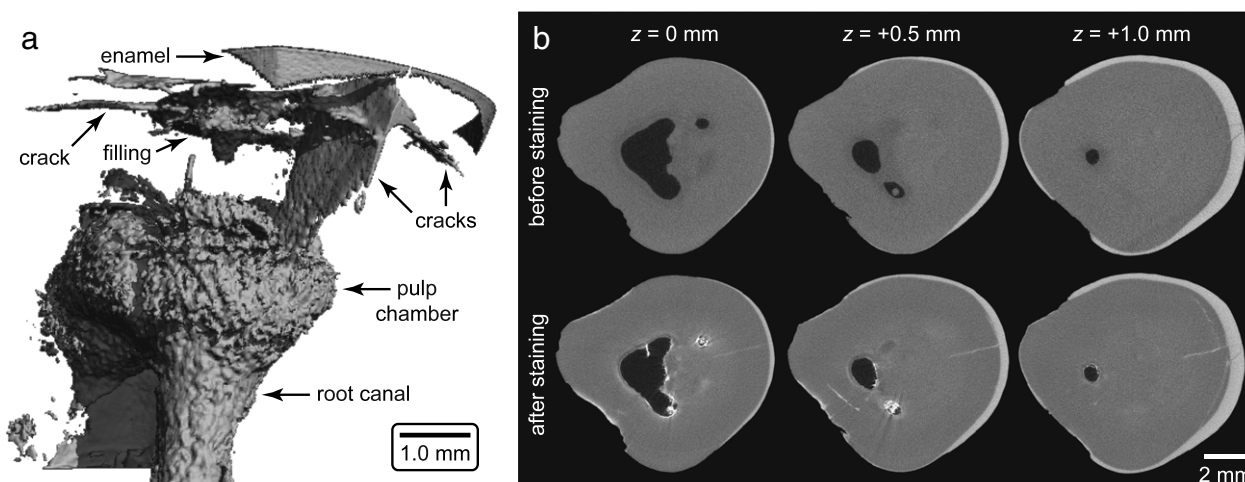


Fig. 2 – An extracted human molar which showed evidence of cracks upon visual inspection was imaged by micro-CT. (a) A segmented, three-dimensional micro-CT reconstruction revealed internal surfaces stained by BaSO₄ precipitation, including the root canal, pulp chamber and cracks, and other features exhibiting the highest x-ray attenuation, including the enamel and a composite filling. Note that the majority of the enamel crown of the tooth has been excluded from the image at the top. (b) Grayscale images of transverse cross-sections showed enhanced contrast for the detection of dentinal cracks after staining by BaSO₄ precipitation compared to the same image cross-section prior to staining.

relative to dentin. The BaSO₄ contrast agent enabled the detection of dentinal cracks that were otherwise not able to be detected using a standard benchtop micro-CT instrument at 10 μm resolution (Figs. 1(b), (c), and 2(b)). The total crack length measured at the free surface in the elephant dentin compact-tension specimens was unchanged before and after staining, which suggests that the staining process did not introduce artifactual damage. Furthermore, the absence of BaSO₄ within cracks during subsequent electron microscopy also enabled discrimination of artifactual cracks that formed during specimen preparation, for example during dehydration, from cracks that formed *in vivo* or during *in vitro* mechanical loading.

The new methods demonstrated in this study are expected to be very useful for *in vitro* scientific studies investigating the mechanical behavior of teeth, including the etiology and morphology of dentinal cracks. Insightful quantitative measurements of crack dimensions, volume, density, orientation, etc. can be subsequently obtained by applying common methods of image analysis to acquired micro-CT data. Such measurements would be extremely tedious, if not impossible, using conventional two-dimensional (2-D) histological techniques. Moreover, even in a highly controlled fracture test, crack length and branching, for example, may exhibit significant 3-D spatial variation that would be otherwise undetected by observation of the specimen surface or any single 2-D cross-section (Fig. 1(a)). Finally, additional potential exists for use of this technique *in vivo* for clinical studies, perhaps with some modification to the staining method or contrast agent. Indeed, cone beam micro-CT with 40 μm resolution is under investigation for clinical use (Kalathingal et al., 2007).

Some limitations should be noted for the new methods demonstrated in this study. BaSO₄ precipitation was nonspecific for cracks, including all void spaces such as vasculature and free surfaces. Furthermore, under the conditions of this

study the BaSO₄ contrast agent was difficult to distinguish from the enamel, presumably due to comparable levels of x-ray attenuation. However, cracks in the enamel were readily detected by micro-CT both with and without the use of a contrast agent (Fig. 2(b)) due to the relatively low x-ray attenuation of the fluid space in the crack compared to the enamel. Therefore, the main limitation of the difficulty distinguishing BaSO₄ from enamel, as well as non-specific staining of exterior specimen surfaces with BaSO₄, was the need to remove the outermost voxels using contouring and a segmented, 3-D reconstructions. Last, the barium chloride and sodium sulfate staining solutions may or may not be acceptable within the oral cavity with some modification, warranting future work and potentially limiting this technique to *in vitro* studies.

5. Conclusions

Contrast-enhanced micro-CT was demonstrated *in vitro* to enable non-destructive, 3-D imaging of the presence, morphology and spatial location of dentinal cracks in whole human molars and machined, partially fractured specimens. In both types of specimens, BaSO₄ staining provided enhanced contrast for the detection of cracks that were not able to be detected prior to staining. Backscattered SEM micrographs showed that BaSO₄ was precipitated on the surfaces of dentinal cracks and within adjacent tubules. The new methods demonstrated in this study are expected to be immediately useful for *in vitro* scientific studies investigating the etiology and treatment of dentinal cracks in teeth, and also possess potential for use in clinical studies with further development.

Acknowledgments

This research was partially supported by the U.S. Army Medical Research and Materiel Command (W81XWH-06-1-0196) through the Peer Reviewed Medical Research Program (PR054672).

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmbbm.2009.10.003.

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