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Role of Alcohol on the Fracture Resistance of Dentin

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Role of Alcohol on the Fracture Resistance of Teeth

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ABSTRACT

Healthy dentin, the mineralized tissue that makes up the bulk of the tooth, is naturally hydrated *in vivo*; however, it is known that various chemical reagents including acetone and ethanol can induce dehydration and thereby affect its properties. Here, we seek to investigate this in light of the effect alcohol can have on the mechanical properties of dentin, specifically by measuring the stiffness, strength and toughness of dentin in simulated body fluid and scotch whisky. Results indicate that chemical dehydration induced by the whisky has a significant beneficial effect on the elastic modulus, strength and fracture toughness of dentin. Although this makes teeth more resistant to fracture, the change in properties is fully reversible upon rehydration. This effect is considered to be associated with increased cross-linking of the collagen molecules from intermolecular hydrogen-bonding where water is replaced with weaker hydrogen-bond forming solvents such as alcohol.

KEYWORDS: dentin, fracture resistance, alcohol, toughening, R-curves

INTRODUCTION

Dentin represents the principal load-bearing material in teeth. It is a hydrated bio-composite of type-I mineralized collagen fibers and nanocrystalline hydroxyapatite, with ~45 vol.% of carbonated apatite mineral (~5 nm thick crystallites), ~30 vol.% type-I collagen fibers (typically 50-100 nm diameter), and aqueous fluid as the remaining 25%. Its most distinctive microstructural feature is 1-2 μm diameter cylindrical tubules (the remnant channels used by odontoblastic cells during tissue formation) that run from the dentin-enamel junction into the interior pulp chamber; collagen fibers form a matte-like network perpendicular to these tubules (Ten Cate, 1994). About 75% of the dentinal fluid is believed to lie within the tubules; the rest is distributed within the intertubular matrix (van der Graaf and Ten Bosch, 1990).

Water is vital in developing and maintaining the structure of the molecules comprising the collagen fibers. It forms a highly ordered inner hydration layer which creates hydrogen-bonds along the underlying peptide chains (Chapman and McLauchlan, 1969; Chapman *et al.*, 1971; Lazarev *et al.*, 1992; Ramachandran and Chandrasekharan, 1968). It also forms hydrogen-bonded “bridges” which further contribute to the structure of collagen by forming intra- and inter-chain links within molecules, along with intermolecular bridges between neighboring triple helices (Bella *et al.*, 1994; Bella *et al.*, 1995).

Certain polar solvents, such as acetone and methanol, are known to chemically dehydrate dentin by replacing the water bonded to the collagen. This behavior is of interest because polar solvent-based adhesive monomers are often used in clinical dentistry to help achieve micromechanical retention of resin composites (Nakabayashi and Pashley, 1998).

Such dehydration causes shrinkage of the tissue, and has also been reported to increase the tensile moduli and strength of dentin (Maciel *et al.*, 1996; Pashley *et al.*, 2001; Pashley *et al.*, 2003). Indeed, our recent studies have shown that the fracture resistance, i.e., toughness, of fully mineralized dentin is also increased by the presence of such solvents, specifically acetone, methanol and ethanol (Nalla *et al.*, 2005). This suggests that dehydration by alcohol may actually strengthen teeth. Accordingly, in the present study we examine whether 86-proof scotch whisky can have a similar effect on the mechanical properties of dentin.

MATERIALS & METHODS

Materials

Elephant dentin, from fractured shards of tusk from an adult male elephant (*Loxodonta africana*), obtained in accordance with IRB protocols for Lawrence Berkeley National Laboratory, was used for the study as it is similar to human dentin in composition, microstructure and mechanical properties; the tubules in elephant dentin are somewhat more elliptical in shape (Raubenheimer *et al.*, 1990) and the peritubular cuffs comparatively smaller. The use of this material permitted the testing of much larger sample sizes.

Deformation behavior testing

To evaluate the stiffness and strength properties of dentin, bending strength tests were conducted. Beams of dentin, ~1.65 x 2.9 x 20 mm ($N=5$), were sectioned such that their length was nominally parallel to the long axis of the tubules, and soaked in a 86-proof scotch whisky (Black & White, James Buchanan and Co., London, U.K.) for ~24 h at room temperature. The beams were loaded to failure (displacement rate=0.015 mm/s)

under three-point bending (center-to-end loading span=7.62 mm) using a servo-hydraulic testing machine (MTS 810, MTS Systems Corp., Eden Prairie, MN), while the loads and load-line displacements were monitored. These data were analyzed to assess differences in the deformation behavior in terms of the initial stiffness (reflective of Young's modulus) and ultimate (bending) strength.

Fracture toughness testing

To measure the fracture toughness of dentin, compact-tension, C(T), specimens ($N=5$), were machined from the shards with specimen thicknesses of ~1.7-2.7 mm, widths of ~12.3-17.1 mm and initial notch lengths of ~3.5-4.3 mm, oriented such that crack growth was perpendicular to the long axis of the tubules and the crack plane was in the plane of the tubules; further details are given in our previous studies (Kruzic *et al.*, 2003; Nalla *et al.*, 2004). The specimens were dehydrated prior to actual testing by soaking in the whisky for 24 h at room temperature. Crack resistance-curve (R-curves) were then measured while continuously irrigated with whisky. This approach involves measurement of the crack resistance as a function of crack extension, $K_R(\Delta a)$, and has been shown by ourselves and others to be the most appropriate means of evaluating the fracture toughness of mineralized tissues such as bone and dentin (Kruzic *et al.*, 2003; Malik *et al.*, 2003; Nalla *et al.*, 2004; Pezzotti and Sakakura, 2003; Vashishth *et al.*, 1997). Specimens were loaded at a displacement rate of ~0.015 mm/s using an MTS 810 testing machine, until the onset of cracking from the notch. At this point, the sample was unloaded by 10-20% of the peak load to record the sample compliance at the new crack length. This process was repeated at regular intervals until the end of the test, at which point the compliance and loading data were analyzed to determine fracture resistance, K_R ,

as a function of Δa ; crack lengths, a , were calculated from the load-line compliance data using standard compliance calibrations (Saxena and Hudak Jr., 1978), while periodically correcting for any errors arising from crack bridging (Kruzic *et al.*, 2003; Nalla *et al.*, 2004). The data were compared with that for ethanol (200-proof alcohol) and water (Hanks' Balanced Salt Solution, HBSS) (Nalla *et al.*, 2005), and analyzed statistically using the non-parametric Kruskal-Wallis test. After testing, crack paths were examined using optical microscopy (Olympus STM-UMS, Olympus America Inc., Melville, NY) and three-dimensional synchrotron x-ray tomography at the Advanced Light Source (Berkeley, CA). The latter technique was performed using monochromatic 16 keV x-rays, with the tomographic data converted into three-dimensional images using the Fourier-filtered back-projection algorithm; full details are described elsewhere (Kinney *et al.*, 2001; Kruzic *et al.*, 2003).

“Dehydrated/ Rehydrated/ Dehydrated” testing

To understand the change in toughness with hydration and dehydration, “dehydrated/rehydrated/dehydrated” testing was performed on specimens ($N=3$) previously used for R-curve testing. An R-curve test was started in whisky (first dehydrated step), interrupted after some crack extension, and the specimens dried out in ambient air for 24 h. The samples were then rehydrated in HBSS for 24 h and tested while being continuously irrigated with HBSS (rehydrated step). After further crack extension, the samples were again dried out in ambient air for 24 h, dehydrated for 24 h in whisky and tested while being continuously irrigated with whisky (second dehydrated step).

RESULTS

Deformation behavior

To investigate the effect of a commonly consumed scotch whisky, the deformation properties of dentin, specifically elastic and plastic yielding behavior, were first evaluated by testing three-point bending specimens soaked in whisky; results were compared with identical tests in water, specifically HBSS, and reagent-grade ethanol. Resulting load-displacement data (Fig. 1) revealed that both the initial stiffness, which is reflective of Young's modulus, and the bending strength were markedly enhanced due to dehydration in whisky; the stiffness increased some 75-100% and the strength ~40-50% compared to hydrated dentin. These values, however, were still 10-20% lower than reported previously for pure (200-proof) ethanol (Nalla *et al.*, 2005).

Resistance curve (R-curve) behavior

The fracture toughness properties of dentin were also found to be enhanced by the presence of whisky. R-curves were used to quantify this by measuring the critical stress intensities both to initiate cracks and to sustain subsequent crack growth. For dentin soaked in whisky, cracks grew stably from the notch for up to 4-6 mm of crack extension; the resulting R-curves (Fig. 2a) can be seen to display a steep rise in toughness over the initial 1-2 mm of crack growth, followed by a flat "plateau" region of nearly constant fracture resistance. Qualitatively, such behavior was similar in all three environments. Quantitatively, three measures of the fracture resistance, the crack-initiation toughness (the initial point on the R-curve), the crack-growth toughness (the slope of the R-curve) and steady-state ("plateau") toughness, were extracted from these data. As with the strength and stiffness (Figs. 2b-d), there were significant differences. Although there was only a small change in the initiation toughness, all other measures of the toughness were significantly higher for dentin dehydrated with whisky, as compared to hydrated dentin;

differences in the growth and plateau toughness were statistically significant ($p < 0.05$). However, values measured in whisky were again lower than for pure ethanol.

“Dehydrated/rehydrated/dehydrated” behavior

These results clearly indicate that alcohol can significantly enhance the fracture resistance of teeth by increasing both the strength and toughness of dentin. However, to further understand these changes, we also performed R-curve tests where we changed the extent of hydration during the test (“dehydrated/rehydrated/dehydrated” tests). Results (Fig. 3) reveal a remarkable effect; whatever benefits that whisky confers in increasing the strength and toughness of dentin, is removed on rehydration. Indeed, the effect of the whisky appears to be completely reversible, as the elevated strength and toughness can be re-established upon re-exposure to whisky.

Crack-path trajectories

Using optical microscopy and synchrotron x-ray tomography, discontinuous crack paths were observed in both whisky and water environments with extensive evidence of crack bridging from “mother” and “daughter” crack configurations (Fig. 4a). The bridges, which tomography (Fig. 4b) verified as being three-dimensional and not just a surface phenomenon, were comprised of unbroken material, often up to several hundred micrometers in size, which spanned the crack behind the crack tip. Such “uncracked-ligament” bridges are primarily formed along the crack path by the imperfect linking of microcracks (“daughter” cracks) that initiate ahead of the tip of the main (“mother”) crack, invariably at the tubules, as shown previously by Kruzic *et al.* (2003) and Nalla *et al.* (2004).

DISCUSSION

This work has shown how whisky, and alcohol in general, can markedly enhance the fracture toughness of dentin, but also that this toughening effect is fully reversible when the dentin is rehydrated with water. A key to understanding this is to appreciate the prime source of the fracture resistance of dentin, which is identified with crack bridging phenomena. The presence of the bridges implies that subsequent cracking will necessitate a higher driving force, as the bridges holding the material together will take up part of the energy applied to drive the crack forward, i.e., the main crack tip will no longer experience the entire applied driving force (“crack-tip shielding”) and the material will appear tougher. Indeed, such uncracked-ligament bridging, has been identified as a potent toughening mechanism in bone as well as in dentin (Nalla *et al.*, 2004). Its presence naturally leads to R-curve behavior, as once the crack starts to grow, more bridges form in the crack wake such that the fracture resistance increases with crack extension. However, with continued crack extension, eventually bridges way behind the crack tip will fail, owing to the larger crack-opening displacements there, and a steady-state is reached whereby bridges are formed at the crack tip at the same rate they are destroyed in the wake (Kruzic *et al.*, 2003); this results in a constant fracture resistance with crack extension, as evidenced by the “plateau” toughness region in our R-curve data. So what role does whisky play in this mechanism? Firstly, it is likely that the increased stiffness and strength of dentin exposed to polar solvents has its genesis in additional hydrogen-bonds between adjacent collagen peptide chains within the collagen fibers (Pashley *et al.*, 2003). Water forms hydrogen-bond bridges across adjacent chains, and when it is replaced with a weaker hydrogen-bond forming solvent like ethanol, fewer of

the hydrogen-bonding sites are occupied by the solvent; additionally, the structure of the collagen molecule is likely to be disrupted from the loss of the hydration layer and change in bonding patterns. The resulting increase in direct collagen-collagen hydrogen-bonding between molecules due to dehydration then leads to a stiffer and stronger material, as shown by the load-deformation behavior.

The higher stiffness and strength of the dentin in whisky associated with increased collagen-collagen hydrogen-bonding in turn leads to stiffer and stronger crack bridges than in hydrated dentin. We believe that it is this enhanced ability of the crack bridges to sustain loads that is the source of the increased fracture toughness of dentin in whisky. This notion is consistent with our experiments that show that these changes in fracture properties are reversible, as the breaking and re-formation of hydrogen-bonds would be both a relatively easy and reversible process.

It is interesting to note that whereas the elastic modulus is the same whether dentin is vacuum-dried or solvent-dried, vacuum-drying decreases the toughness while alcohol drying increases the toughness. In Kruzic *et al.*, (2003), we observed that crack blunting occurred in hydrated dentin, but was absent in air and vacuum-dried dentin. Such blunting decreases the driving force of a dominant crack by reducing the stress intensity at the crack tip; it further facilitates bridge formation, which leads to a rising R-curve in normal hydrated dentin. Rising R-curves are not seen in air-dried dentin, consistent with the absence of crack-tip blunting, but are seen in alcohol-dehydrated dentin, implying that blunting occurs in alcohol. The feature common to both hydrated and alcohol-saturated dentin is the presence of fluid. We conjecture that a fluid layer must facilitate the blunting

of cracks in dentin, consistent with the significantly different toughness properties in alcohol-driven vs. vacuum-dried dentin.

In summary, we have used alcohol to probe fundamental questions in restorative dentistry and mineralized tissue research. For many years, there has been clinical debate over whether endodontically-restored teeth are more “brittle” than untreated teeth. Many have argued that endodontically-restored teeth are less moist, and therefore would be more prone to brittle fracture (Helfer *et al.*, 1972). Indeed, several attempts have been made to measure the moisture content of teeth, often with contradictory findings. Here, we demonstrate that partial removal of water, and its replacement with whisky, actually increases the fracture resistance of the tooth. However, since removal of water by testing *in vacuo* conversely lowers the toughness of dentin (Kruzic *et al.*, 2003), we believe that it is not water *per se* that may be important; rather, it appears that the presence of a fluid is more critical for the proper mechanical function of the tooth.

Finally, our observations that changes in water content can have pronounced effects on both the elastic and fracture properties of a mineralized tissue indicate that processes that occur at the molecular level are important in regulating mechanical behavior at all length scales. Thompson *et al.* (2001) have recently shown that collagen properties at the molecular length scale in bone are affected by changes in the fluid chemistry; this work demonstrates that these changes can indeed influence fracture, but over much larger length scales.

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LIST OF FIGURES

Figure 1: Typical load-displacement data ($N=5$ each) for chemically dehydrated dentin (in 200-proof ethanol and 86-proof scotch whisky) and hydrated dentin (in HBSS), based on three-point bending tests. The initial (elastic) portion of the load-displacement curve is a measure of the stiffness and is reflective of the Young's modulus; the maximum point on each curve is a measure of the ultimate bending strength. It is apparent that by soaking the dentin samples in whisky or alcohol to dehydrate them, leads to a significant increase in the stiffness and strength of the dentin.

Figure 2: (a) Fracture resistance data for dentin tested with continuously irrigation in whisky ($N=5$), expressed in terms of crack-resistance curves (R-curves), measured on compact-tension, C(T) specimens. Each data point type represents a separate sample. The shaded regions on the R-curves indicate similar data obtained for hydrated dentin and dentin dehydrated in pure ethanol. The bar graphs (mean $\pm$ S.D.) show a significant increase in (b) crack initiation, (c) crack growth and (d) steady-state ("plateau") fracture toughness for dentin dehydrated in whisky and ethanol, as compared to hydrated dentin. Differences in the growth and plateau toughness were statistically significant ($p < 0.05$).

Figure 3: Representative results for the "dehydrated/rehydrated/dehydrated" tests ($N=3$) for whisky expressed in terms of the fracture toughness R-curve behavior. These experiments give a clear indication how the toughness drops when dehydrated dentin is exposed to water (HBSS), and that the fracture toughness of dentin is significant higher in whisky. However, they also provide clear evidence that the effect is completely reversible.

Figure 4: (a) An optical micrograph showing the development of crack bridging in dentin dehydrated using whisky. Microcracks ("daughter cracks"), initiated primarily at tubules, form ahead of the main crack ("mother crack"); their inability to link perfectly with the main crack leads to regions of uncracked material which spans the crack. Such "uncracked ligaments" carry load that would otherwise be used to drive the crack, and

thereby act to tough the dentin. (b) A three-dimensional x-ray tomography reconstruction of a section of the crack in (a), showing the three-dimensional nature of the uncracked-ligament bridging. The nominal direction of crack growth is indicated in both cases.

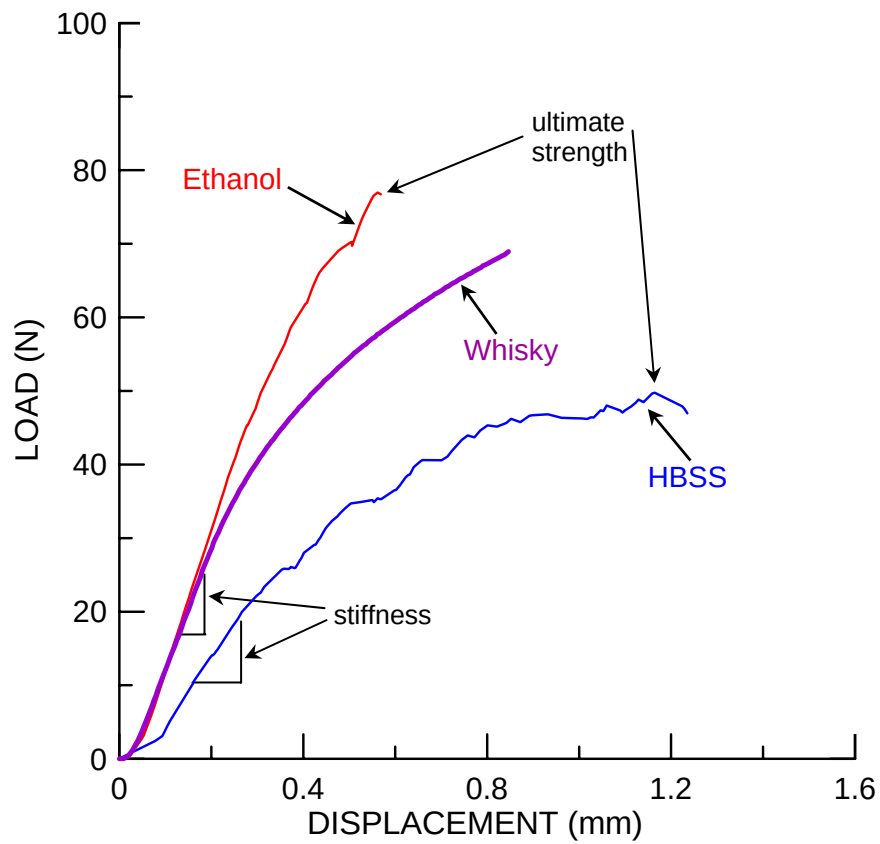
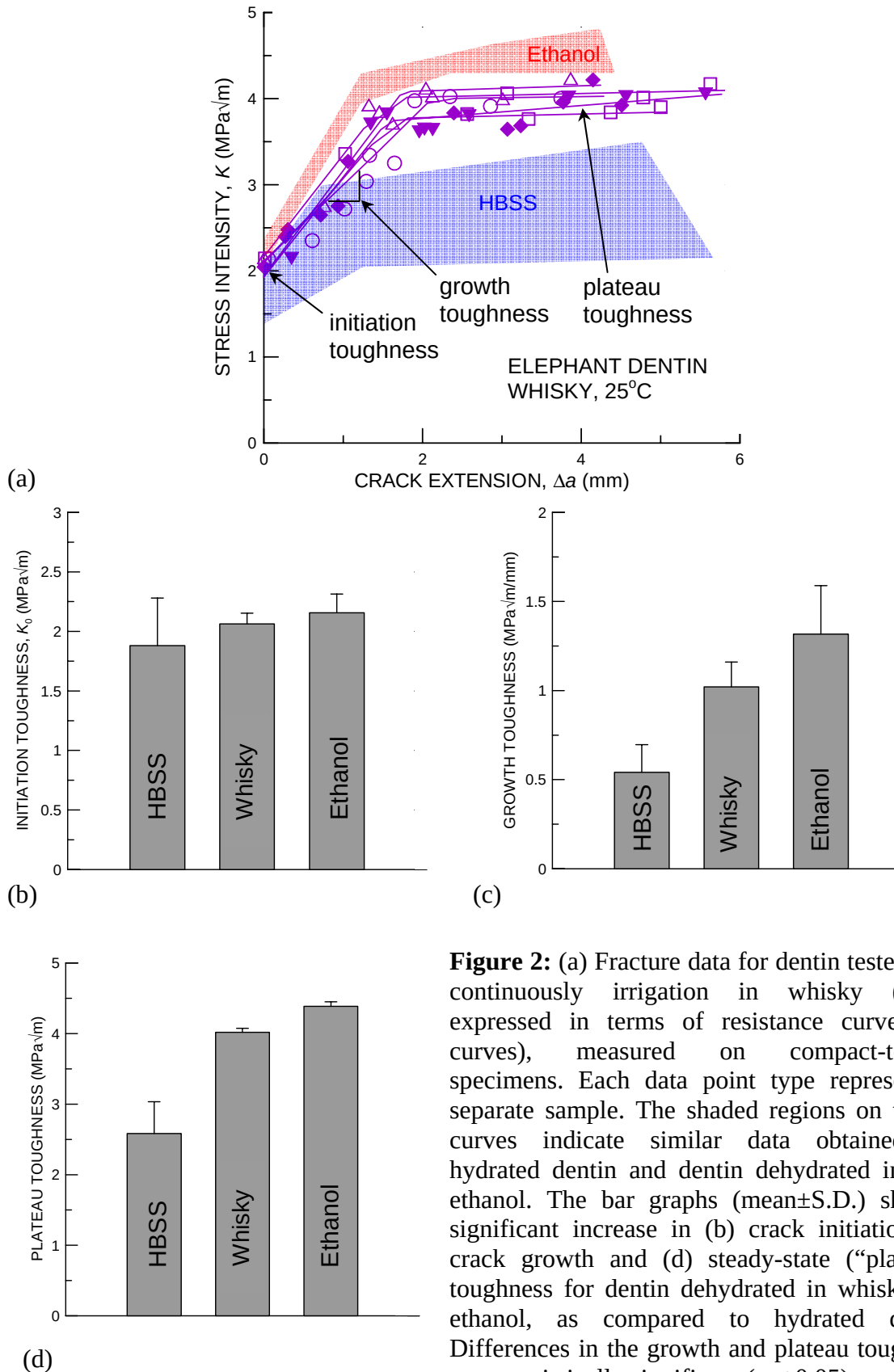


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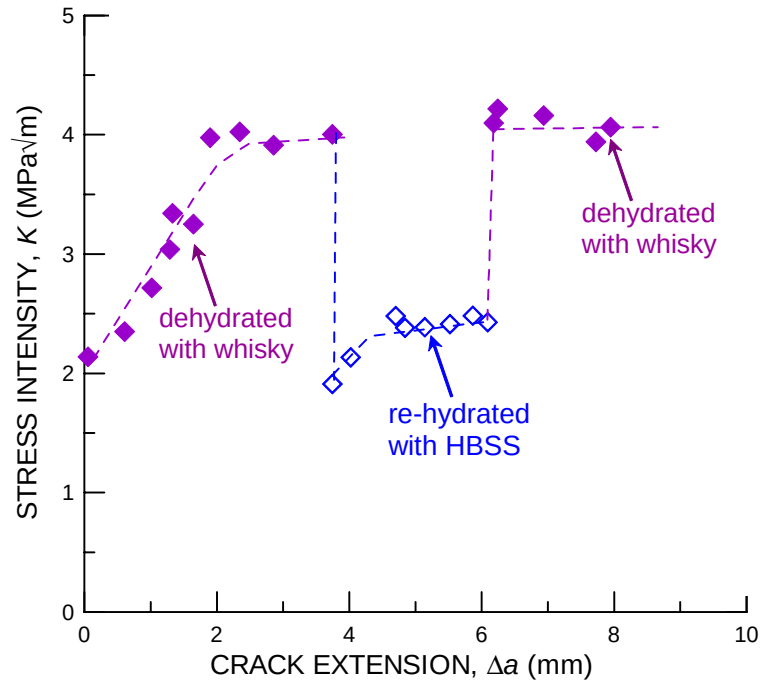


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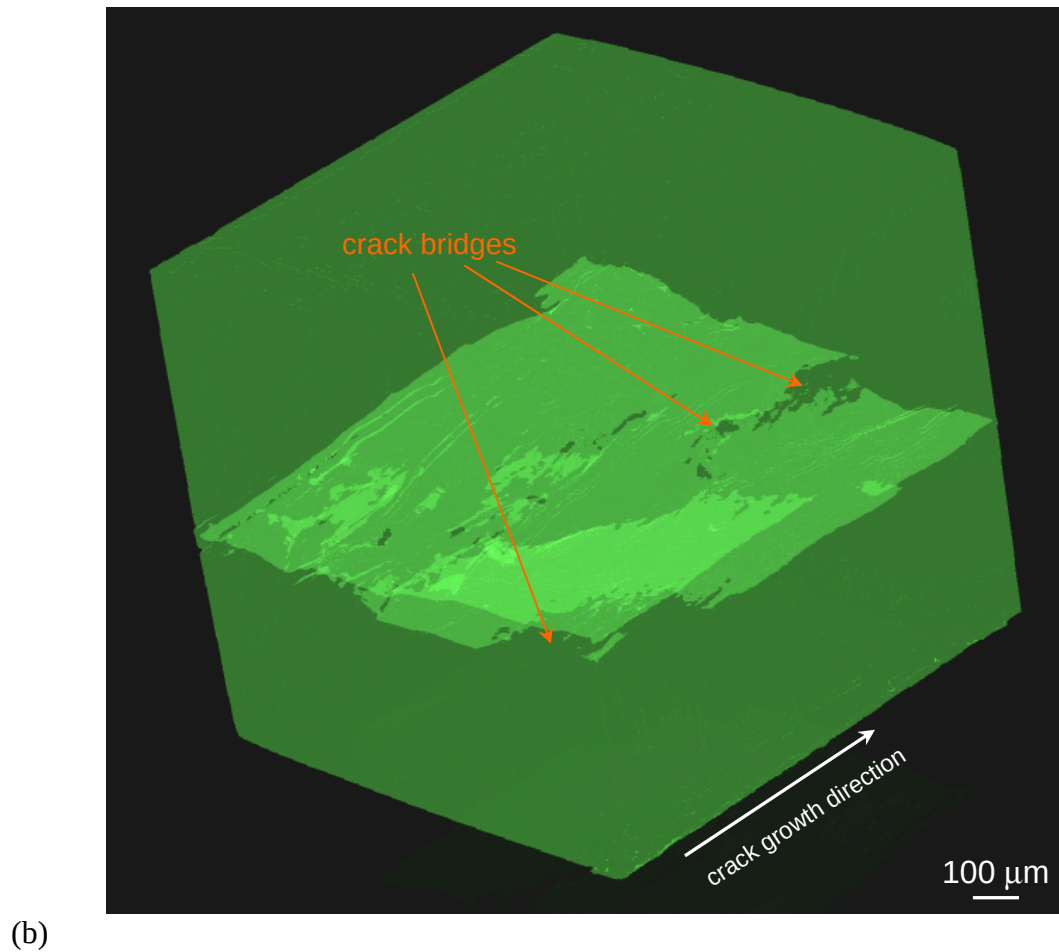
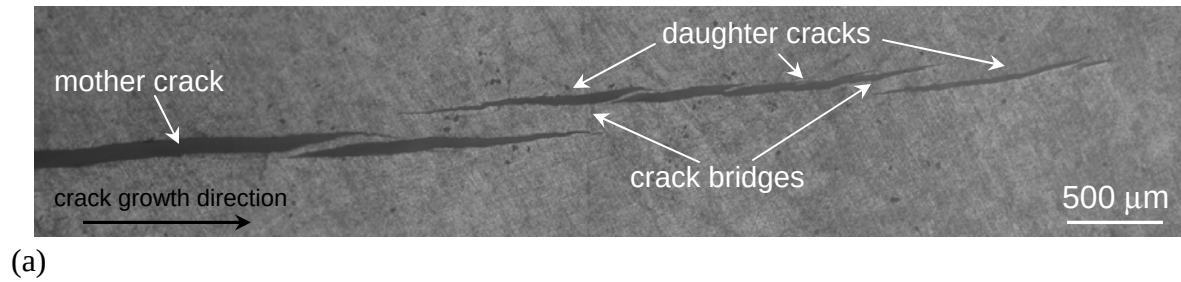


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