Published in final edited form as:

Mater Res Soc Symp Proc. 2008 ; 1132: . doi:10.1557/PROC-1132-Z09-05.

Effects on Hardness and Elastic Modulus for DSS-8 Peptide Treatment on Remineralization of Human Dental Tissues

Chia-Chan Hsu¹, Hsiu-Ying Chung^{1,2}, Elizabeth Marie Hagerman³, Wenyuan Shi⁴, Jenn-Ming Yang¹, and Ben Wu^{1,3}

¹ Department of Materials Science and Engineering, University of California, Los Angeles, Los Angeles, CA 90095, U.S.A

² Department of Materials Science and Engineering, Feng Chia University, Taichung 407, Taiwan, Republic of China

³ Department of Bioengineering, University of California, Los Angeles, Los Angeles, CA 90095, U.S.A

⁴ School of Dentistry, University of California, Los Angeles, CA 90095, U.S.A

Abstract

Dental remineralization may be achieved by mediating the interactions between tooth surfaces with free ions and biomimetic peptides. We recently developed octuplet repeats of aspartate-serine-serine (DSS-8) peptide, which occurs in high abundance in naturally occurring proteins that are critical for tooth remineralization. In this paper, we evaluated the possible role of DSS-8 in enamel remineralization. Human enamel specimens were demineralized, exposed briefly to DSS-8 solution, and then exposed to concentrated ionic solutions that favor remineralization. Enamel nano-mechanical behaviors, hardness and elastic modulus, at various stages of treatment were determined by nanoindentation. The phase, microstructure and morphology of the resultant surfaces were characterized using the grazing incidence X-ray diffraction (GIXD), variable pressure scanning electron microscopy (VPSEM), and atomic force microscopy (AFM), respectively. Nanoindentation results show that the DSS-8 remineralization effectively improves the mechanical and elastic properties for demineralized enamel.

Keywords

Enamel; Peptide; Nanoindentation; Remineralization

1. Introduction

Human enamel is a highly mineralized extracellular matrix including 96 % inorganic mineral and 4 % organic material and water. The inorganic enamel is a crystalline hydroxyapatite (HA) with average thickness of 5 nm, and average crystal length of 20 nm. Although the hydroxyapatite crystal exhibits anisotropic properties, enamel achieves its remarkable hardness due to the three dimensional complex microarchitecture.^{1,2}

Saliva and oral fluid continuously promote the deposition of minerals into the dental hard tissues, enamel and dentin. If the rate of acid-induced dissolving process caused by the oral

bacteria is kinetically greater than that for mineral deposition process, dental caries occurs.^{3,4} Currently, the most common clinical treatment for dental caries is filling the diseased tissue with material that does not perform the biological functions of native tissue. However, a preferable therapy is self-reconstruction by means of applying biomaterials to promote regeneration of healthy biological tissue.

Dentin phosphoprotein (DPP) is a highly acidic protein due to the high concentrations of phosphoserine (45–50 %) and aspartic acid (35–38 %) containing up to 90 % serine and is the most abundant non-collagenous extracellular matrix (ECM) component.⁵ There are two kinds of repetitive sequences for nucleotide corresponding to 244 residues at the carboxyl terminal of DPP. One of the DPP sequence containing the blocks of aspartate-serine-serine (DSS) repeating between 3 and 14 times has been modeled to promote the formation of hydroxyapatite.⁶ The DSS-8 peptides, octuple repeats of aspartate-serine-serine, are the most active in the mediation of biologically directed mineral growth.^{7–10} Therefore, the DSS-8 peptides were used in this study to investigate the effects on microstructures, hardness and elastic modulus for human enamel in the remineralization process.

2. Materials and methods

2.1 Materials preparation

Human adult molars were collected and washed in distilled water. The teeth were embedded in epoxy resin (Leco, St. Joseph, MI) and then the cross-sections along the parallel median plane were cut using a 331-CA diamond blade (Struers, Cleveland, OH). Subsequently, the surfaces were polished using 800 grit and 1200 grit silicon carbide (SiC) sand papers, followed by 3 μm , 1 μm and 0.5 μm Al_2O_3 and 0.06 μm colloidal silica in sequence. Finally, the samples were individually sonicated in deionized (DI) water for 5 minutes.

2.2 Nucleation experiments

Half of the samples were demineralized with 35 % phosphoric acid for 30 seconds and then rinsed thoroughly with DI water; the other half were left untreated. All samples were then sonicated for five minutes to remove excess debris left from cutting, polishing and demineralization processes. As for the remineralization process, samples were exposed to 12.5 or 62.5 μM DSS-8 dissolved in HEPES buffer solution (pH 7.0) for 30 minutes and afterward immersed in a simulated body fluid (SBF)^{11–13} at 36.8 °C for 24 hours (pH 6.8) to accelerate the nucleation of hydroxyapatite (HA) crystals.

2.3 Nanoindentation measurements

Nanoindentation testing was carried out in an MTS XP nanoindenter (MTS Nano Instrument, Oak Ridge, TN) with a three-sided Berkovich diamond tip. Before testing, both the diamond tip and the nanoindenter were calibrated using fused silica. Each nanoindentation testing consists of two segments, loading and unloading segments. The hardness and elastic modulus were calculated using Equation [1], [2] and [3] developed by Oliver and Pharr¹⁴ with a continuous stiffness measurement (CSM) technique, which involves applying a very small oscillation force to the loading force at high frequency.

$$H = \frac{P_{\max}}{A} \quad [1]$$

$$\frac{1}{E_r} = \frac{1 - \nu^2}{E} + \frac{1 - \nu_i^2}{E_i} \quad [2]$$

$$E_r = \frac{S}{\sqrt{A}} \frac{\sqrt{\pi}}{2\beta} \quad [3]$$

Here $P_{\max}$ is the maximum load, A is the contact area, E is the elastic modulus and is the Poisson's ratio of the specimen; E_i and ν_i are the elastic modulus and Poisson's ratio for the diamond indenter. E_r is the reduced modulus which depends on the slope of the upper-portion of the load-displacement unloading curve (S), and β is a shape constant for the indenter. From the oscillation of the resulting depth signals, the contact stiffness is continuously measured so that the values of hardness and elastic modulus can be obtained along the varying indent depth. The amplitude of the force oscillation is small enough that it does not affect the deformation process.

Due to the organic matrix phase, the mechanical behavior of human enamel is significantly sensitive to the strain rate;¹⁵ therefore, efforts were made to ensure the materials were loaded at constant strain rate (CSR), a target CSR of 0.05 s^{-1} set in the loading segments for all tests.

3. Results and Discussions

3.1 Indentation hardness and Young's modulus of enamel

Influences of DSS-8 peptide on native enamel—First, the native enamel was remineralized and the results show that the hardness significantly decreases from 4.42 to 2.37 GPa after remineralization treatment with $12.5 \mu\text{M}$ DSS-8 peptide. However, the hardness only shows a small decrease, from 4.42 to 4.21 GPa, when the enamel was remineralized containing no DSS-8 peptide. The elastic modulus of remineralized enamel shows the similar trend as found in hardness, decreasing from 86.90 to 35.12 GPa after remineralization treatment with $12.5 \mu\text{M}$ DSS-8 peptide and a less decrease from 86.90 to 66.53 GPa remineralized without containing DSS-8 peptide. Therefore, the adding of DSS-8 peptide into the remineralization process did not improve the mechanical and elastic properties of native human enamel; contrarily, it destroys the bonding and thus results in the lower hardness and elastic modulus compared to those for native enamel.

Influences of DSS-8 peptide on demineralized enamel—In order to simulate the effect of DSS-8 peptide upon the decayed teeth, the native enamels were demineralized for 30 seconds, followed by the remineralization treatments. The hardness of enamel decreases from 4.42 to 0.32 GPa after demineralization with 30 seconds. This is because some minerals have been dissolved and organic materials such as proteins between the HA fibers existing in native enamel have been etched away in the demineralization treatment process.

¹⁶ Comparatively, the whole structure becomes less consolidated; therefore, the hardness and elastic modulus of native enamel decrease after demineralization treatment. Then the hardness subsequently increases to 0.79 GPa with additional remineralization containing 12.5 μ M DSS-8 peptide. In contrast, the hardness of enamel remineralized without DSS-8 peptide is 0.66 GPa, lower than that treated with DSS-8 peptide. These differences may be due to many etched minerals in the demineralization treatment process.

The elastic modulus of enamel substantially decreases from 86.90 to 27.25 GPa, a 69 % decrease, with the demineralized treatment for 30 seconds. Following by the remineralization, the elastic moduli are 29.71 and 42.29 GPa for the enamel treated without and with 12.5 μ M DSS-8 peptide, respectively. The results indicate that adding 12.5 μ M DSS-8 peptide in the remineralization process can effectively improve the hardness and elastic modulus for the demineralized enamel.

3.2 SEM and AFM

The surface roughness of native enamel is 65 nm, and then after remineralization treatments, the surface roughness increases to 272 and 347 nm without and with 12.5 μ M DSS-8 peptide, respectively. Additionally, the new-minerals form and accumulate at the middle area. Much of the native enamel structure is etched away and the surface roughness significantly increases from 65 to 2267 nm. Additionally, the boundaries of enamel rods become apparent and a long nanorod-like structure is found inside each enamel rod. Under both treatments, the surfaces become much smoother compared to that of demineralized enamel. The individual nanorods found inside demineralized enamel rods become indistinct after remineralization treatment without DSS-8 peptide. The overall average surface roughness is about 1829 nm and in some local smooth areas it shows a roughness of 246 nm. In contrast, there are fiber-like minerals formed on the demineralized enamel remineralized with 12.5 μ M DSS-8 peptide, whose overall surface roughness is about 1734 nm and 107 nm in some smooth local areas.

3.3 GIXD

The GIXD pattern of native enamel is identical with JCPDS 00-001-1008, hydroxyapatite (HA; $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$).¹⁷ Evidently, there is no preferred orientation for the HA crystals according to the relative intensities of the each peaks in the GIXD patterns. The native enamels remineralized without and with DSS-8 peptides form calcium phosphate carbonate [$\text{Ca}_{10}(\text{PO}_4)_6\text{CO}_3$]¹⁸ and carbonate-apatite [$\text{Ca}_{10}(\text{PO}_4\text{CO}_3\text{OH})_6(\text{OH})_2$]¹⁹, respectively. After demineralization, the main phase found in enamel is still HA compound. The demineralized enamel remineralized without DSS-8 peptides forms carbonate-apatite; however, the demineralized enamel remineralized with 12.5 μ M DSS-8 peptide forms carbonate-apatite and HA. The hardness and elastic modulus of native enamel decrease after remineralized with 12.5 μ M DSS-8 peptide since the surface of native enamel (65nm) is not rough enough to make DSS-8 peptides effectively react with the surface of native enamel. In contrast, the high surface roughness of 2267 nm is beneficial for DSS-8 peptides to bind on the demineralized enamel and this subsequently results in the formations of carbonate-apatite [$\text{Ca}_{10}(\text{PO}_4\text{CO}_3\text{OH})_6(\text{OH})_2$] and HA [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] uniformly covering on the surface.

Thus, the hardness and elastic modulus of demineralized enamel significantly increase after remineralized with 12.5 μ M DSS-8 peptide.

4. Conclusion

Remineralization treatments effectively improve the mechanical and elastic properties for demineralized enamel but not for native enamel. The result may be due to the difference in surface roughness and DSS-8 binding affinity among the native and demineralized enamel surfaces. The hardness and elastic modulus for the demineralized enamel remineralized with DSS-8 peptide are higher than those remineralized without containing DSS-8 peptide. In addition, both the type and morphology of the newly grown minerals dominate the resulting mechanical and elastic properties.

Acknowledgments

The authors would like to express their gratitude to Dan Yarbrough and Jian He, School of Dentistry, University of California, Los Angeles, for help with DSS-8.

References

1. Landis WJ, Song MJ, Leith A, McEwen L, McEwen BF. Mineral and organic matrix interaction in normally calcifying tendon visualized in three dimensions by high-voltage electron microscopic tomography and graphic image reconstruction. *J Struct Biol.* 1993; 110:39–54. [PubMed: 8494671]
2. Nanci, A. Ten cate's oral histology. St. Louis: Mosby Elsevier; 2003.
3. Suga, S.; Watabe, N. Hard tissue mineralization and demineralization. Tokyo: Springer; 1992.
4. Cuy JL, Mann AB, Livi KJ, Teaford MF, Weihs TP. Nanoindentation mapping of the mechanical properties of human molar tooth enamel. *Arch Oral Biol.* 2002; 47:281–91. [PubMed: 11922871]
5. Stetler WGS, Veis A. Bovine dentin phosphophoryn: calcium ion binding properties of a high molecular weight preparation. *Calcif Tissue Int.* 1987; 40(2):97–102. [PubMed: 3105840]
6. George A, Bannon L, Sabsay B, Dillon JW, Malone J, Veis A, Jenkins NA, Gilbert DJ, Copeland NG. The carboxyl-terminal domain of phosphophoryn contains unique extended triplet amino acid repeat sequences forming ordered carboxyl-phosphate interaction ridges that may be essential in the biomineralization process. *J Biol Chem.* 1996; 271 (51):32869–73. [PubMed: 8955126]
7. He G, Dahl T, Veis A, Geroe A. Nucleation of apatite crystals in vitro by self-assembled dentin matrix protein 1. *Nat Mater.* 2003; 2(8):552–8. [PubMed: 12872163]
8. Sikes, CS.; Yeung, ML.; Wheeler, AP. In surface reactive peptides and polymers: discovery and commercialization. Washington: ACS; 1991.
9. Qiu SR, Wierzbicki A, Orme CA, Cody AM, Hoyer JR, Nancollas GH, Zepeda S, Yoreo JJD. Molecular modulation of calcium oxalate crystallization by osteopontin and citrate. *Proc Natl Acad Sci.* 2004; 101(7):1811–5. [PubMed: 14766970]
10. Bigi A, Boanini E, Rubini K, Gazzano M. Hydroxyapatite nanocrystals modified with acidic amino acids. *Eur J Inorg Chem.* 2006; 23:4821–6.
11. Papanearchou NI, Leventouri T, Kis AC, Hotiu A, Anderson IM. Effect of simulated body fluid on the microstructure of ferrimagnetic bioglass-ceramics. *Mater Res Soc Symp Proc.* 2005; 839:371–6.
12. Kokubo T, Takadama H. How useful is SBF in predicting in vivo bone bioactivity. *Biomaterials.* 2006; 27:2907. [PubMed: 16448693]
13. Murphy WL, Monney DJ. Bioinspired growth of crystalline carbonate apatite on biodegradable polymer substrata. *J Am Chem Soc.* 2002; 124(9):1910–7. [PubMed: 11866603]
14. Oliver WC, Pharr GM. Review: Measurement of hardness and elastic modulus by instrumented indentation: Advances in understanding and refinements to methodology. *J Mater Res.* 2004; 19(1):3–20.

15. Zhou J, Hsiung LL. Biomolecular origin of the rate-dependent deformation of prismatic enamel. *Appl Phys Lett*. 2006; 89:51904.
16. He LH, Swain MV. Influence of environment on the mechanical behavior of mature human enamel. *Biomaterials*. 2007; 28:4512–20. [PubMed: 17659336]
17. JCPDS No. 00-001-1008. International Center for Diffraction Data; Newton Square, PA: 2003.
18. JCPDS No. 00-035-0180. International Center for Diffraction Data; Newton Square, PA: 2003.
19. JCPDS No. 00-004-0697. International Center for Diffraction Data; Newton Square, PA: 2003.