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Research Paper

# Effects of peptide concentration on remineralization of eroded enamel

*Hsiu-Ying Chung*[a,b,*], *Kuo-Chen Huang*[a]

[a]*Department of Materials Science and Engineering, Feng Chia University, Taichung 407, Taiwan*
[b]*Center for Micro/Nano Science and Technology, National Cheng Kung University, Tainan 701, Taiwan*

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ABSTRACT

Promoting remineralization to repair eroded enamel is a promising therapy in clinics. In this study, biocompatible asparagine–serine–serine (NSS) peptide chelates free ions from artificial saliva through charged functional groups, and subsequently form nano-hydroxyapatite crystals to partially repair erosive lesions. The nanomechanical properties, cross-sectional microstructure, types of deposited minerals, and subsurface microstructure of enamel at various treatment stages were characterized by nanoindentation, scanning electron microscopy (SEM), X-ray diffraction (XRD), and transmission electron microscopy (TEM), respectively. The results revealed that the nanohardness and elastic modulus of eroded enamel increase with peptide concentration, particularly for the 3NSS peptide system. In contrast, the structure of the 5NSS peptide is larger and longer, leading to increasing difficulty in penetrating to the deep acid-eroded regions; therefore, the remineralization effect was restricted to the top enamel surface. The 3NSS peptide with high concentration promoted the formation of smaller, finer, and staggered nanohydroxyapatite crystals. The enamel remineralized with a 100 μM 3NSS exhibited the highest degree of nanohardness recovery (34%), resulting from subsurface crystalline regrowth.


## 1. Introduction

Human enamel is a highly mineralized extracellular matrix including 96 wt% inorganic mineral and 4 wt% organic material with water. The unique three-dimensional nano-architecture of apatite crystals contributes to the remarkable mechanical properties and the biological protection.

Demineralization refers to the dissolution of calcium and phosphate ions from tooth to saliva, while remineralization refers to the mineral precipitation into tooth structure. Enamel is relatively stable in the healthy oral environment, although there is a dynamic equilibrium between demineralization and remineralization at the tooth-pellicle and plaque–saliva interfaces (Aoba, 2004). Since the net mineral gain induced by saliva is minute, early caries lesions occur when bacterial plaque, cariogenic microflora, or dietary carbohydrates are present (Houte, 1994; Dorozhkin and Epple, 2002), where the dissolution rate of calcium phosphates is greater than the recombination rate.

The consumption of acidic soft drinks causes demineralization and softening of human enamel; thereby, dental erosion is a result of the dissolution of tooth tissue by acids

*Corresponding author at: Department of Materials Science and Engineering, Feng Chia University, Taichung 407, Taiwan. Tel.: +886 4 24517250x5305; fax: +886 4 24510014.
*E-mail addresses:* hychung@mail.fcu.edu.tw, hychung@fcu.edu.tw (H.-Y. Chung).

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of non-bacterial origin. The carboxylic acids chemically absorb onto the enamel surface and dissolve $Ca^{2+}$ ions out of the hydroxyapatite surface (Yoshida et al., 2001). Acids with a concentration that gave an equivalent sensorial acidic taste resulted in dissimilar erosive morphology and nanohardness loss (Beyer et al., 2011). Promoting the remineralization of early small dental erosion would prevent the formation of advanced lesions. The preventive strategies involve the use of casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) nanocomplexes (Reynolds, 1997; Shen et al., 2001; Yamaguchia et al., 2006; Tantbirojn et al., 2008; Hannig and Hannig, 2010), calcium phosphates (Cai and Tang, 2008; Huang et al., 2009), bioglass (Vollenweider et al., 2007; Dong et al., 2011), and peptides (Kirkham et al., 2007; Benesch et al., 2008; Segman-Magidovich et al., 2008; Hsu et al., 2011; Chung et al., 2012).

Previous studies have demonstrated that the numerous repetitive nucleotide sequences of aspartic–serine–serine (DSS) in dentin phosphoprotein (DPP) and the synthetic DSS peptide possess the capability of promoting the formation of hydroxyapatite crystals and further improving the nanomechanical properties of acid-eroded human enamel (George et al., 1996; Hsu et al., 2011; Chung et al., 2012). Additionally, the enamel remineralized using the NSS peptide, a derivative of the DSS peptide, where the $CONH_2$ group replaces the COOH group in Asp amino acid, exhibits a higher degree of recovery compared with that using the DSS peptide (Chung and Li, 2013). Although the peptide is highly biocompatible as a remineralization promoter, the degree of hardness recovery has not achieved an optimum level. In this study, the peptide concentration was increased to investigate whether the degree of recovery can be improved further. Furthermore, the effects of the number of repetitive nucleotide sequences in the peptide on the nanomechanical behaviors and microstructures of remineralized human enamel were also investigated.

## 2. Materials and methods

### 2.1. *Sample preparation*

Twenty adult human third molars were collected after informed consent as approved by the Commission for Medical Ethics of China Medical University. The specimens were selected based on clinical appearance as free from stain, caries or enamel defects, cleaned from debris, and stored in distilled water with 0.5% thymol solution until use. The preparation process of enamel slab was shown in Fig. 1. Each whole tooth was embedded in a long-cure epoxy resin (Leco, St. Joseph, MI, USA), and then transversely sectioned using a water-cooled diamond blade (Accutom 50, Struers, Cleveland, OH, USA). The size of enamel slab was 5 mm (L) × 1 mm (W) × 3 mm (thickness). To obtain smooth surface, the specimens were finally ground with SiC paper (grit 1200–4000; 3 M, USA) and polished with $Al_2O_3$ powder dispersions (particle size ranging from 1 μm to 0.3 μm; Buehler, USA). All polished samples were individually sonicated in distilled water for 5 min to remove residual abrasives.

Enamel specimens were treated with 1 M citric acid (pH ~ 2) for 2 min, and then immediately rinsed with deionized water (Eisenburger et al., 2001; Barbour et al., 2003). To simulate aggressive enamel erosion, this process was repeated five times to create demineralized enamel. Demineralized specimens were randomly assigned to 15 groups. The experimental specimens ($n=10$ for each group) were exposed to the peptide at various concentrations for 60 min. The peptide was dissolved in a HEPES buffer, and the concentration of peptide was adjusted to 25, 50, 100, and 200 μM. Each specimen was then placed in 100 mL artificial saliva at 37 °C for 14 days, and the artificial saliva was renewed every other day. Three peptides, NSS, 3NSS, and 5NSS, representing peptides with the sequences of NSS, NSSNSSNSS, and NSSNSSNSSNSSNSS, were purchased (Kelowna International Scientific Inc., Taiwan) and used in this study. To simulate an oral cavity remineralization environment, we chose artificial saliva rather than a supersaturated simulated body fluid. The artificial saliva was prepared by dissolving 6.84 mM NaCl, 5.37 mM KCl, 5.44 mM $CaCl_2 \cdot 2H_2O$, 4.42 mM $NaH_2PO_4$ $2H_2O$, 0.21 mM $Na_2S \cdot 9H_2O$ and 16.65 mM urea in deionized water (Holland, 1992), and 3.08 mM sodium azide was added to prevent bacterial growth (Gu et al., 2010). The pH value was adjusted to 7.0 with 1 M NaOH. The artificial saliva is supersaturated with regard to the hydroxyapatite (degree of saturation, DS > 1). The specimens ($n=10$) directly incubated in artificial saliva without peptide treatment served as controls.

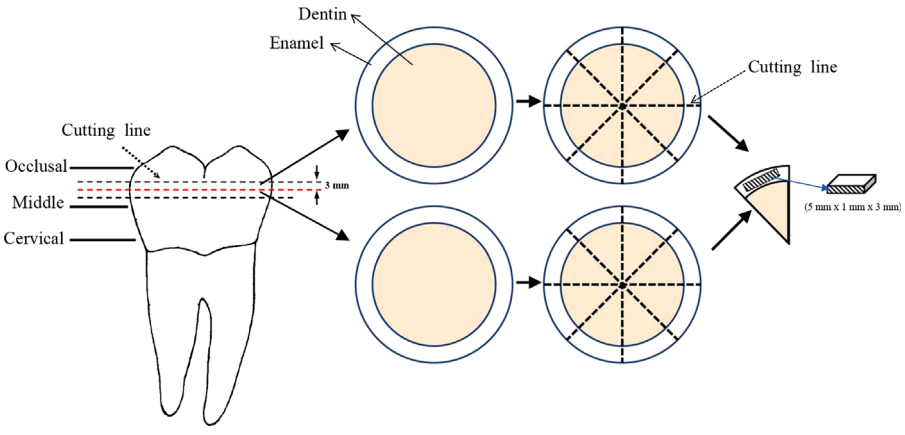


**Fig. 1 – A schematic preparation process of enamel slab.**