

Supporting Information

An engineered dual-functional peptide with high affinity to demineralized dentin enhanced remineralization efficacy *in vitro* and *in vivo*

Meng Li^{1,†}, Yuanyuan Tu^{1,†}, Wanchun Zhu², Menglin Fan¹, Zilin Zhou¹, Zhaohan Yu¹,
Shunhua Wang¹, Yingming Yang¹, Jiyao Li¹, Kunneng Liang^{1,*}

¹State Key Laboratory of Oral Diseases, National Clinical Research Center for Oral
Diseases, Department of Cariology and Endodontics, West China Hospital of
Stomatology, Sichuan University, Chengdu 610041, China

² College of Stomatology, Chongqing Medical University, Chongqing, 401147, China

Key words: dental caries; peptide; non-collagenous proteins; collagen fibrils; binding
capacity; tooth remineralization.

Experimental section

Synthesis of CYP

Peptides were synthesized using Fmoc/tBu strategy on an ABI 431A multiple peptide synthesizer (Applied Biosystems, USA). First, 100 mg Fmoc-Tyr (tBu)-Merrifield resin (Merck KGaA, Darmstadt, Germany) was added into the reactor for treatment of pre-peptide synthesis. Then, various Fmoc-amino acids were gradually added into the synthesator according to the sequence of peptides from the C-terminal (Cys-Gln-Asp-Ser-Glu-Thr-Arg-Thr-Phe-Tyr-Asp-Ser-His-Ala-Lys-Arg-His-His-Gly-Tyr-Lys-Arg-Lys-Phe-His-Glu-Lys-His-His-Ser-His-Arg-Gly-Tyr-OH). The reaction cycle time of each amino acid was 40 minutes. 93, 600 mg Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBop) reagent, 15, 600 mL 1-Hydroxybenzotriazole (Hobt) aqueous solution and 45, 000 mL 4-Methylmorpholine should be added at the same time. After that, add 180, 000 mL piperidine to the deprotecting reaction and 450, 000 mL N, N-Dimethylformamide to the washing reaction each coupling. Completed peptides were cleaved from the resin with trifluoroacetic acid and appropriate scavengers. The precipitation was collected by centrifugation and dried in vacuum. Finally, the crude peptide is about 256 mg.

Crosslinking procedures of collagen gels

To stabilize the reconstituted collagen structures, the crosslinking procedures were conducted: first, a solution containing 0.3 mol/L 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide (EDC) and 0.06 mol/L N-hydroxysuccinimide (NHS) was prepared. The

pH of the EDC/NHS solution was adjusted to 5.9 using 2-morpholinoethane sulphonic (MES) powder. Then, the collagen grids were incubated in 80 μL of the EDC/NHS solution for 4 h, and then rinsed with deionized water and air-dried.

Results

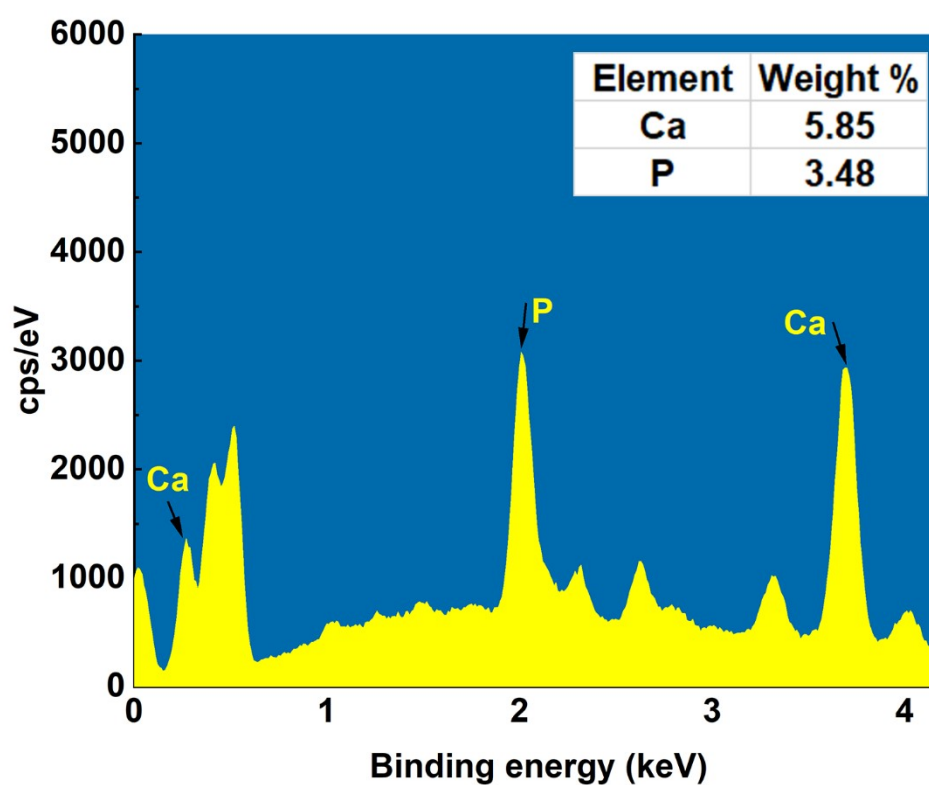


Figure S1. EDS spectrum of the 14th day of titanium plate treated with CYP and the ratio of Ca/P is 1.68.

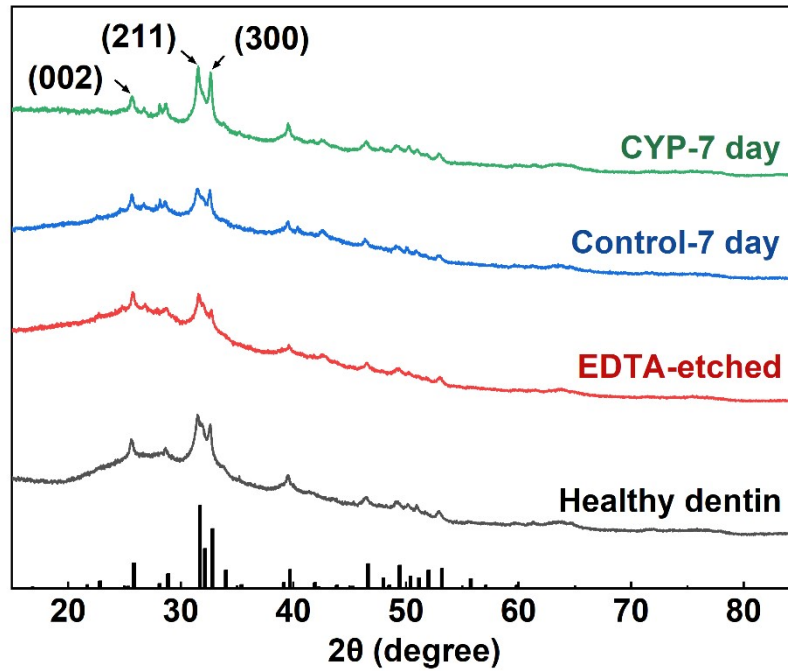


Figure S2. XRD patterns of the 7th day of healthy dentin, EDTA-etched dentin, dentin specimens treated with CYP and deionized water during the remineralization cycle.

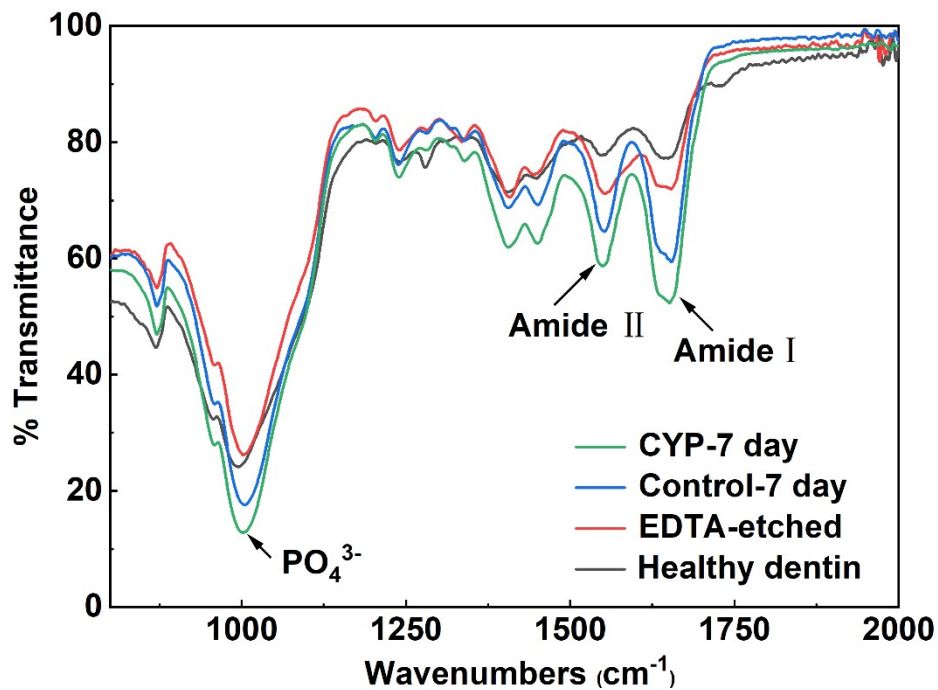


Figure S3. FTIR spectra of the 7th day of healthy dentin, EDTA-etched dentin, dentin specimens treated with CYP and deionized water during the remineralization cycle.

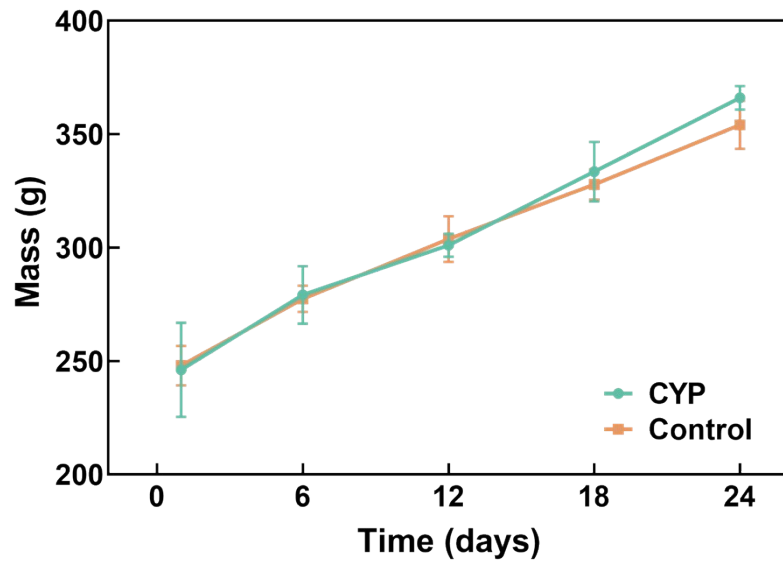


Figure S4. Body weights of rats during the experimental period.

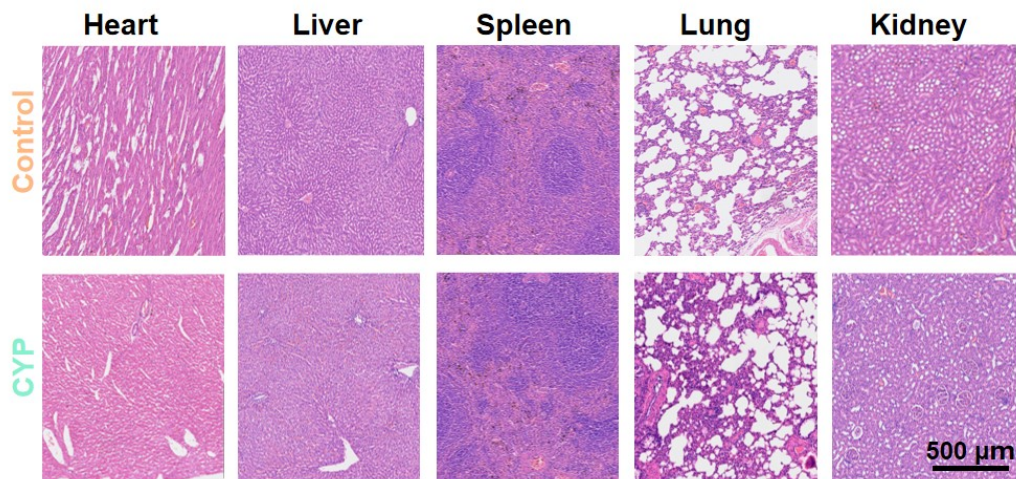


Figure S5. HE staining images from heart, liver, spleen, lung, and kidney tissue slices of control and CYP group.