Substrate-directed zinc phosphate nanodeposition for durable dentin bonding: One-step interfacial dehydration and proteolysis resistance

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Supplementary Information

Supplementary Materials and Methods

Effect of buffer pH on the morphology of demineralized dentin matrix

Prepare 30 mL of 50 mM HEPES buffer, divide it into three 10 mL aliquots, and adjust the pH of two aliquots to 5.0 and 4.5, respectively. Dentin slices were subjected to conventional acid etching, treated with the respective solutions for 30 s, rinsed for 20 s, and followed by graded ethanol dehydration and scanning electron microscopy (SEM) examination (Nova Nano 450, Thermo FEI, Waltham, CZ).

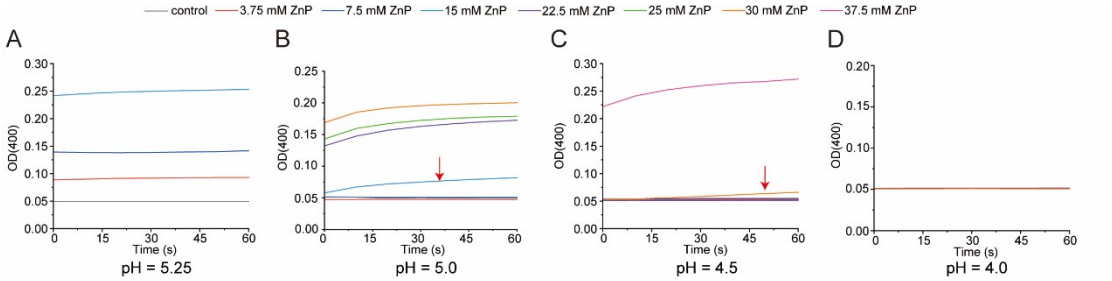
In vitro evaluation of protein hydrolysis activity

To evaluate the inhibitory effect of nano-ZnPs, a type I collagenase degradation assay was performed. The experiment was divided into four groups:

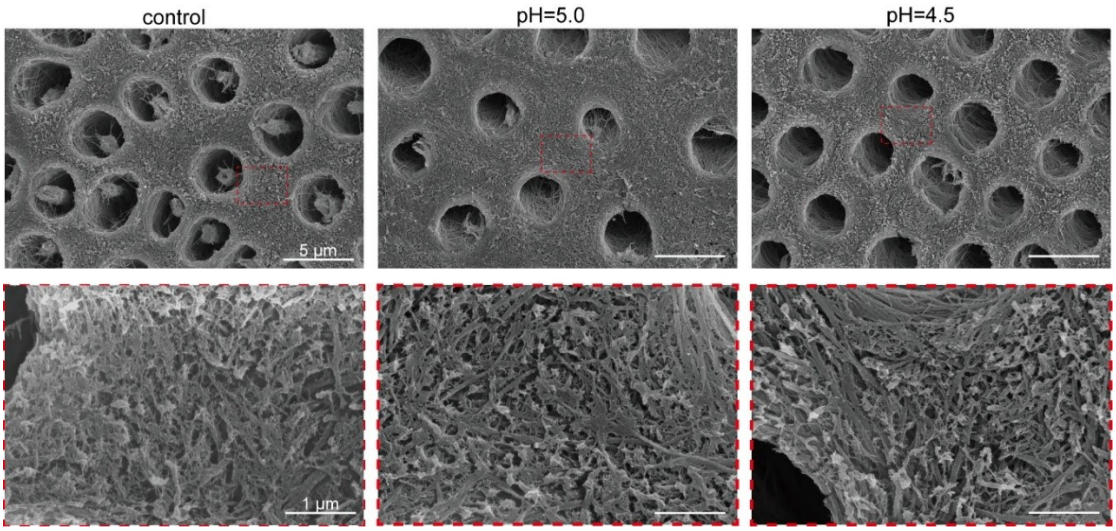
1. control group: 5 mL of 66 μg/mL enzyme solution (from clostridium histolyticum, ≥125 CDU/mg solid, Beyotime, Shanghai, CHN).
2. Zn^{2+} group: 5 mL of 30 mM $ZnSO_4$ mixed with 5 mL of 132 μg/mL enzyme solution.
3. ZnP group: 5 mL of 30 mM $ZnSO_4$ was mixed with 5 mL of 20 mM K_2HPO_4 for 30 s, centrifuged, and the precipitate was resuspended in 10 mL of 66 μg/mL enzyme solution.
4. Zn^{2+} + ZnP group: 2.5 mL of 60 mM $ZnSO_4$ was mixed with 2.5 mL of 40 mM K_2HPO_4 for 30 s, followed by the addition of 5 mL of 132 μg/mL enzyme solution.

Dentin slices were conventionally acid-etched and then incubated in the respective solutions at 37 °C for 2, 4, or 6 hours. After incubation, samples were thoroughly rinsed and followed by graded ethanol dehydration and SEM examination (Nova Nano 450, Thermo FEI, Waltham, CZ).

Supplementary Figures

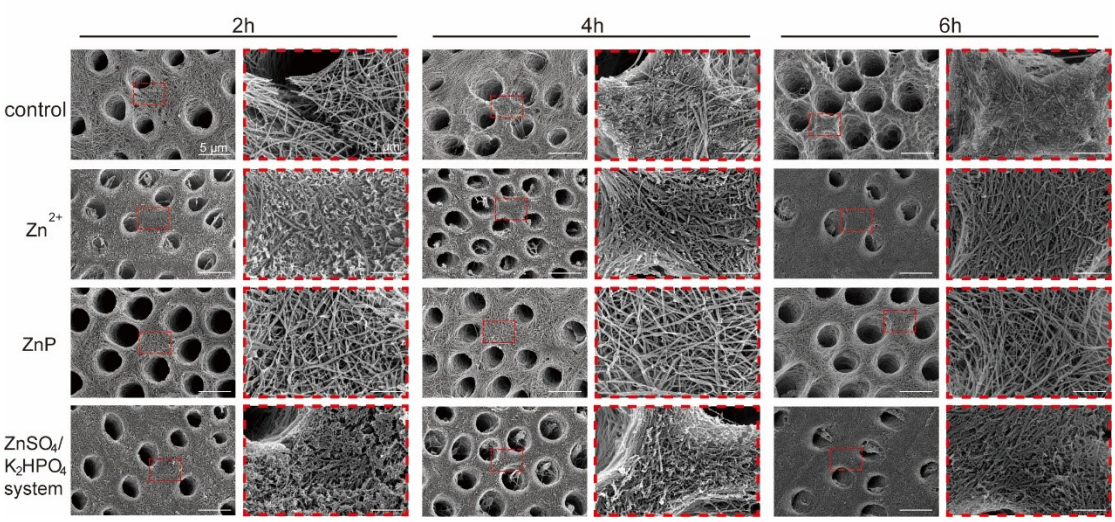


Supplementary Fig. 1 Screening of kinetically suitable Zn^{2+}/phosphate precursor conditions by monitoring OD changes. Optical density (OD) traces were recorded to evaluate turbidity onset in kinetically unstable mixtures of $ZnSO_4$ and K_2HPO_4 across a range of concentrations and pH values. (**A**) At pH 5.25, all concentration groups exhibited instantaneous turbidity, indicating rapid precipitation. (**B**) At pH 5.0, no turbidity developed in the 3.75 and 7.5 mM groups; the 15 mM group showed a gradual OD increase within 30 s, whereas the 22.5–30 mM groups precipitated immediately. (**C**) At pH 4.5, the 3.75–22.5 mM groups remained optically clear; the 30 mM group exhibited a delayed OD rise within 30 s, while the 37.5 and 45 mM groups showed immediate turbidity. (**D**) At pH 4.0, no precipitation occurred at any tested concentration.

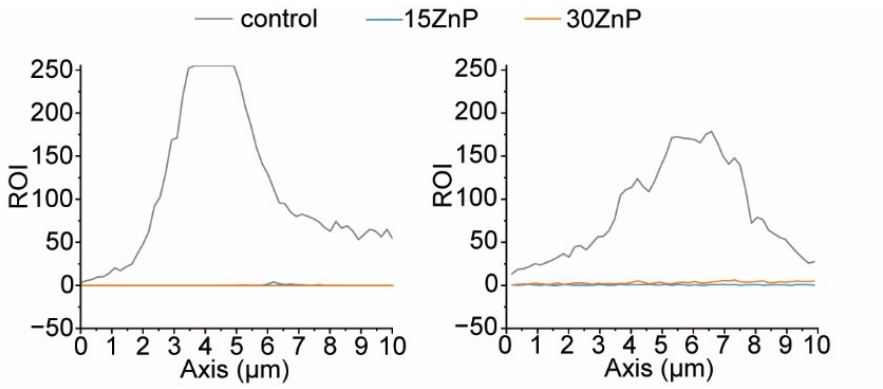


Supplementary Fig. 2 The effect of pH in HEPES solutions on demineralized dentin. Representative SEM images of demineralized dentin after treatment with 50 mM HEPES solutions at different pH values, with a magnified view of the region

highlighted by the red rectangle. Scale bars: 5 μm (low magnification) and 1 μm (high magnification).



Supplementary Fig. 3 Inhibitory effect of Zn^{2+} and nano-ZnPs on exogenous collagenase. SEM images showing the surface morphology of demineralized dentin matrix after treatment with different enzyme solutions for 2, 4, and 6 hours. Scale bars: 5 μm (low magnification) and 1 μm (high magnification).



Supplementary Fig. 4 Semi-quantitative analysis of *in situ* zymography fluorescence at the resin-dentin interfaces. Fluorescence intensity profiles across the hybrid layer were quantified for each experimental group. The left panel presents longitudinal fluorescence distributions for the Single Bond 2 system, and the right panel shows the corresponding analysis for the Spectrum Bond system.