Information

A Novel Fluorescent Adhesive-assisted Biomimetic Mineralization

Zhe Wang,[a, b] Yan Ouyang,[a] Zhifang Wu,[a,b] Leiqing Zhang,[a,b] Changyu Shao,[c] Jiayan Fan,[a,b] Ling Zhang,[a,b] Ying Shi,[a,b] Zihuai Zhou,[a,b] Haihua Pan,[d] Ruikang Tang,[c,d] and Baiping Fu,*[a,b]

a：Department of Prosthodontics, Hospital of Stomatology Affiliated to Zhejiang University School of Medicine, Hangzhou 310006, Zhejiang, China;

b：Key Laboratory for Oral Biomedical Research of Zhejiang Province, Hangzhou, 310020, Zhejiang, China

c：Center for Biomaterials and Biopathways, Department of Chemistry, Zhejiang University, Hangzhou 310027, Zhejiang, China.

d：Qiushi Academy for Advanced Studies, Zhejiang University, Hangzhou 310027, Zhejiang, China.

Supplementary Methods

1. Preparation of reconstituted type I collagen fibrils

Type I collagen solution (3 mg/mL, 8.33 μL) derived from rat tail was mixed with 0.5 mL of collagen assembling solution (0.1wt% NaN3, 50 mM glycine, 200 mM KCl) to obtain 50 μg/mL collagen solution and kept at room temperature for 20 min. Then, 3 μL of droplets were dropped onto 400-mesh carbon coated Ni grids (T11023N, Xinxingbairui, China) and kept in a 100% humidity chamber at 37℃ for at least 8 hrs. Thereafter, cross-linking of the reconstituted collagen fibrils was performed with 0.05 wt % of glutaraldehyde for 1 h. Finally, the collagen-coated grids were rinsed with triply distilled water and dried in air.[1] Two grids were randomly selected and stained with 1% uranyl acetate for 15 s in order to analyze

the reconstituted type I collagen fibrils by TEM.

2. Preparation of demineralized enamel and dentin disks

Twenty human premolar teeth and 20 third molar teeth were collected with the patients' informed consent. The protocol in this study was approved by the Institutional Ethics Committee. The buccal enamel disks of premolar and coronal dentin disks of the third molar teeth were prepared as 3mm×3mm×1mm with a slow-speed saw (Isomet 1000, Buehler) under cooling water.[2] After the enamel and dentin surfaces were polished with 600-grit silicon carbide paper under running water, they were etched with 37% phosphoric acid, enamel for 30 s and dentin for 10 s, in order to obtain the demineralized enamel and dentin disks.

3. Preparation of the enamel and dentin samples for TEM

The remineralized enamel and dentin samples were fixed in 2.5% glutaraldehyde at 4 °C overnight before they were each rinsed with 1 mL of phosphate buffer saline (PBS) for three times. The samples were dehydrated in an ascending series of ethanol (30%, 50%, 70%, 90%) for 15 min, absolute ethanol and acetone for 20 min, respectively. Then they were embedded in epoxy resin and kept in 70°C oven overnight.[3] Ultrathin sections (90~110 nm) of enamel and dentin samples were prepared and analyzed by TEM (JEM-1230, JEOL, Tokyo, Japan) with selected area electron diffraction (SAED), and high resolution transmission electron microscopy (HRTEM, JEM-2100F, JEOL, Tokyo, Japan) with Mapping.

4. CLSM test for enamel and dentin samples after 24 hrs and 4 wks

4.1 Preparation of the enamel and dentin samples for CLSM

Another four enamel/dentin samples (n=4) were prepared and treated with the experiment adhesive as the same as the above-mentioned. After the enamel/dentin samples were stored in the artificial saliva for 24 hrs and 4 wks, they were embedded in epoxy resin as the same as described in preparation for TEM samples. The samples were cut into two pieces vertical to the remineralization layer

with the slow-speed saw under cooling water. The cross-sectioned surfaces of the samples were polished with a series of silicon carbide papers (360, 500, 800, 1000, 1200, 2400, 4000 grit) (SAPHIR 360, Germany).[4] Finally, the samples were analyzed by CLSM (IX81-FV1000，Olympus，Tokyo, Japan).

4.2 Results and discussion of CLSM

The fluorescent mineralizing adhesive penetrates into approximately 20-30 μm of the demineralized enamel and approximately 50-60 μm of the demineralized dentin after 24 hrs and 4wks of storage in artificial saliva. Thus, ACP nanoparticles can infiltrate the demineralized enamel and dentin along with the adhesive, which might be beneficial to induce teeth mineralization. Regretably, the boundaries between the original teeth and the remineralized layer were not detected by CLSM due to the nanoscale remineralization in this study.

Furthermore, the adhesive layer and hybrid layer at the interfaces of adhesive-demineralized enamel/dentin could not be distinguished in this study. The adhesive layer and hybrid layer of the enamel samples (Fig. S5 a, b) were thicker and brighter than those of dentin (Fig. S5 c, d). It might be attributed to the monomer-Ca salts formed on the adhesive-enamel surface, which resulted from the chemical interaction of the acidic monomer of the adhesive with enamel hydroxyapatite. Furthermore, calcium has been demonstrated to increase the fluorescence at the adhesive-dentin/enamel interface since calcium can be chelated with calcium-chelator such as sodium fluorescein.[4] On the contrary, there were nearly completely demineralized collagen fibrils on the demineralized dentin surface, therefore, few monomer-Ca salts could be formed on the surface of the demineralized dentin. We added CLSM images of enamel/dentin samples after 24 hrs and 4 wks of storage in artificial saliva in support information（Fig. S5）.

5. Preparation of the enamel and dentin samples for XRD

The de- and re-mineralized enamel/dentin samples were prepared and treated with the experiment adhesive as the same as the above-mentioned. The surface of all the samples were determined by XRD diffractometerwith Cu target at 0.154 nm. The acceleration voltage was performed at 40 kV with 40 mA of current.[5]

Supplementary Figures

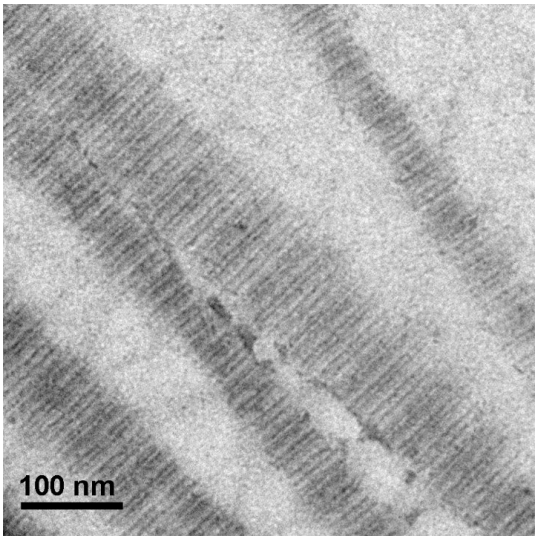

Fig. S1 TEM images of reconstituted type I collagen fibrils stained with uranyl acetate.

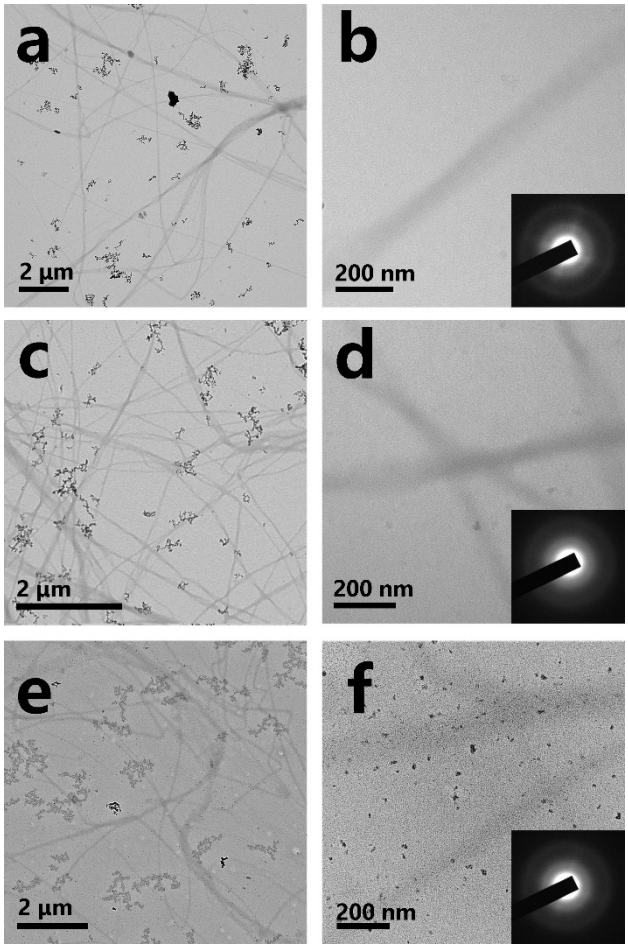

Fig. S2 TEM images of S3 (control group) on type I collagen fibrils for 1, 4, 7 days. Panels b, d, f are the higher magnifications of panels a, c, e, respectively. No obvious mineralization was observed. SAED patterns (insert in panels b, d, f) shows an amorphous state of type I collagen fibrils.

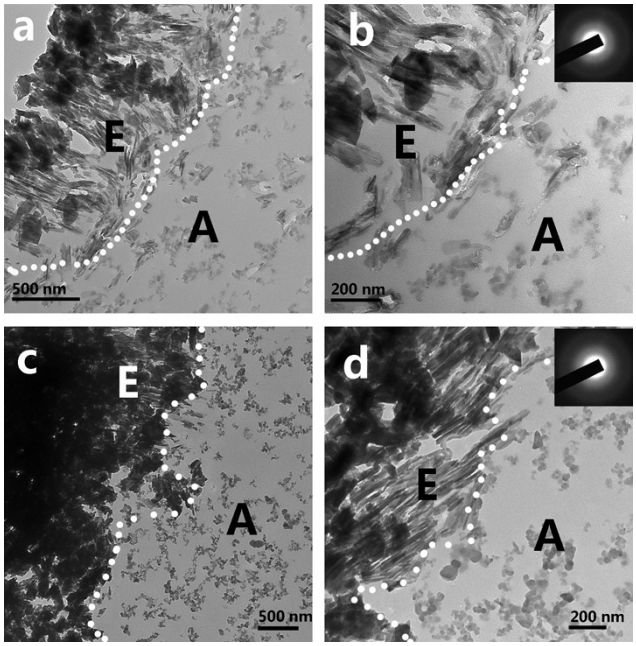

Fig. S3 TEM images of S3 (control group) on demineralized enamel after 2 and 4 wks of storage in artificial saliva. Panels b, d, f are the higher magnifications of the red box areas in panels a, c, respectively. No obvious mineralization could be found. A: adhesive; E: enamel.

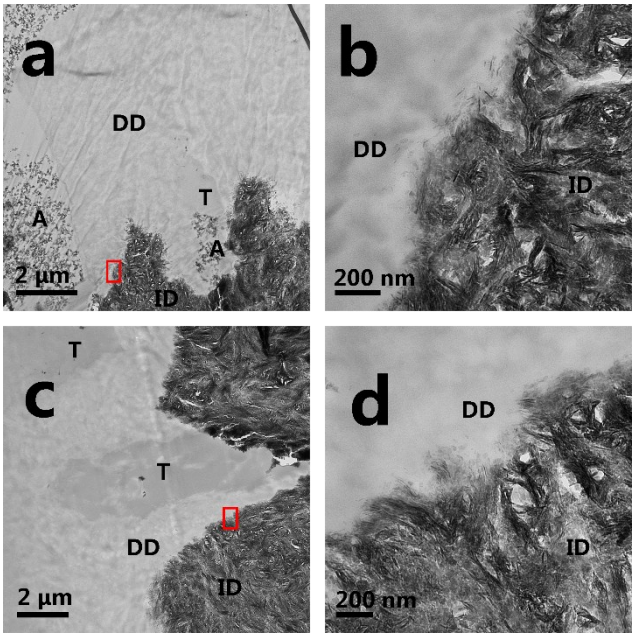

Fig. S4 TEM images of S3 (control group) on demineralized dentin after 2 and 4 wks of storage in artificial saliva. Panels b, d are the higher magnifications of the red box areas in panels a, c, respectively. No obvious mineralization was detectable. A: adhesive; ID: intact dentin; DD: demineralized dentin; T: dentin tube.

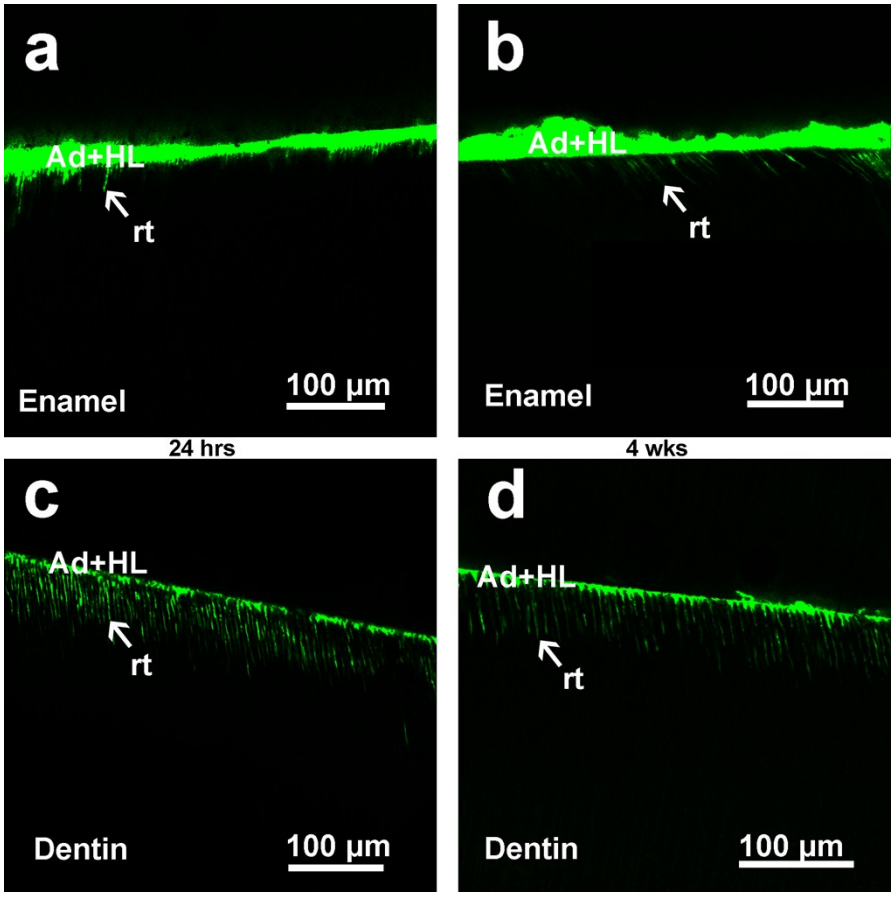

Fig. S5 The CLSM images of the enamel (panels a, b) and dentin (panels c, d) after 24 hrs (panels a, c) and 4 wks (panels b, d) of storage in artificial saliva. The fluorescent mineralizing adhesive penetrates into approximately 20-30 µm of the demineralized enamel (panels a, b) and approximately 50-60 µm of the demineralized dentin (panels c, d). The adhesive layer and hybrid layer (Ad+HL) on the enamel surface (panels a, b) were thicker and brignter than those of dentin (panels c, d). The boundaries between the original teeth and the remineralized layer could not be detected (panels b, d). Ad+HL: adhesive layer and hybrid layer; rt: resin tags.

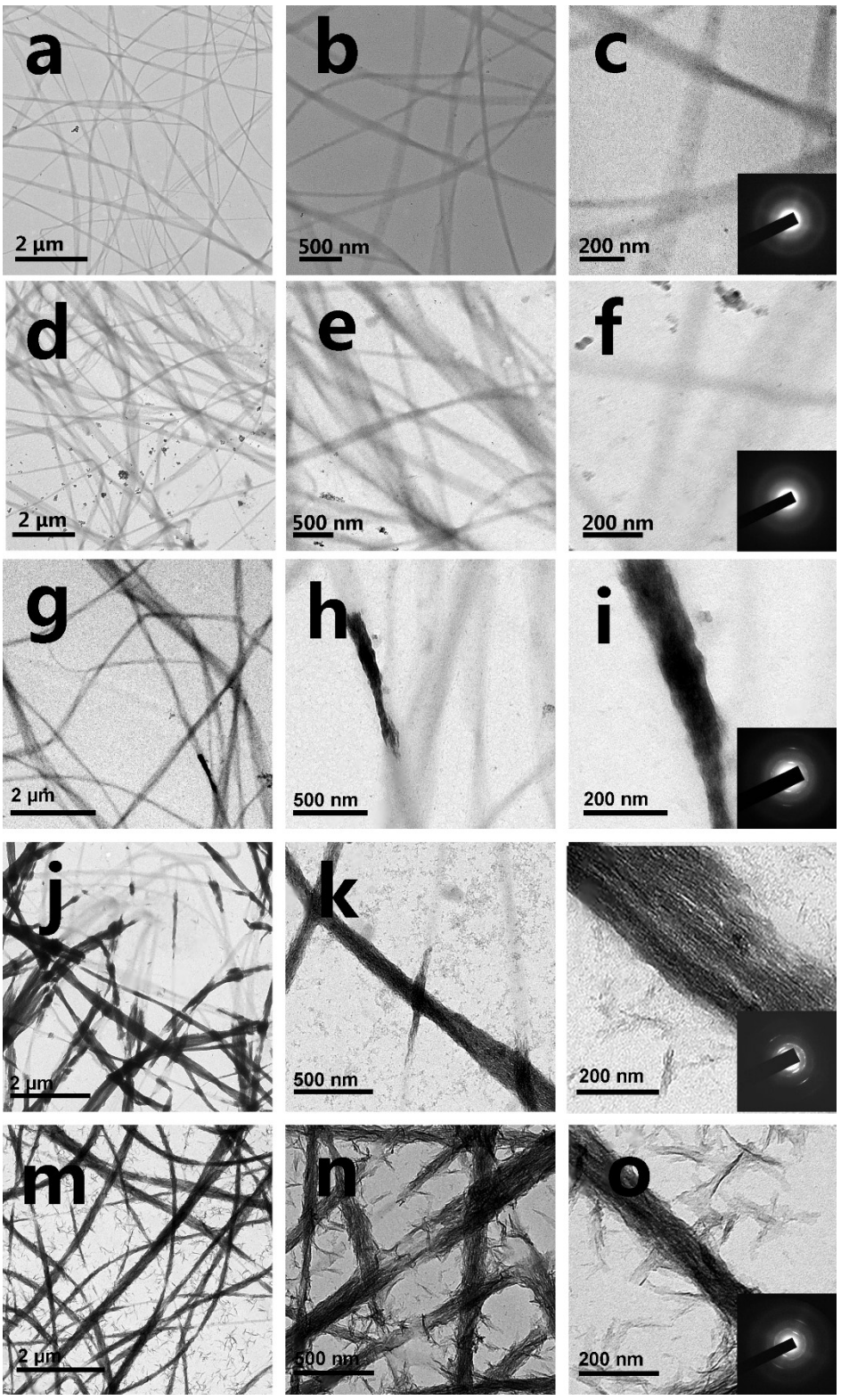

Fig. S6 TEM images of the reconstituted type I collagen fibrils treated with the adhesive S3 added with 0 wt % (panels a-c), 5 wt % (panels d-f), 10 wt % (panels g-i), 15 wt % (panels j-l), and 25 wt % of PAA-ACP nanoparticles (panels m-o) after 7 days of storage in artificial saliva. Panels b, c; e, f; h, i; k, l; n and o are the higher magnifications of panels a, d, g, j and m, respectively. The collagen fibrils were not mineralized in panels a-f (0 wt %, 5 wt % PAA-ACP). The SAED patterns (insets in panels c, f) indicate that the mineral phase Is non-crystalline. A few collagen fibrils were mineralized in panels g-l (10wt% PAA-ACP). Some collagen fibrils were mineralized in panels j-l (15 wt %). Most of collagen fibrils were mineralized in panels m-o (25 wt % PAA-ACP). The SAED patterns (insets in panels i, l, o) indicate that the intrafibrillar mineral phase is apatite.

Supplementary References

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