

Supporting Information

Self-Etch Adhesive as a Carrier for ACP Nanoprecursors to Deliver Biomimetic Remineralization

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SUPPLEMENTARY EXPERIMENTAL SECTION

All the chemical reagents were purchased from Aladdin Reagent Co., Ltd. and were of analytical chemical grade. Triply distilled water was used in this study, and all solutions were filtered through 0.22 μm Millipore film prior to use.

Preparation of a Single-layer Collagen Model. The preparation of a single-layer collagen model referred to ref.¹ Type I collagen solution derived from rat tail (3 mg/mL, Gibco, Invitrogen) was diluted to 50 $\mu\text{g/mL}$ in 0.5 mL of assembling solution (pH 9.2, 50 mM glycine, 200 mM KCl) and kept at room temperature for 20 min. After 3 μL of droplets of the collagen solution were dropped on 400-mesh nickel grids coated with formvar/carbon film (Beijing Zhongjingkeyi Technology Co., Ltd., China), the grids were placed in a Petri dish overnight in a 100% humidity chamber at 37 °C. Collagen cross-linking was performed with 0.05 wt% glutaraldehyde for 1 h. Thereafter, the collagen-coated grids were washed thoroughly with distilled water and dried in air. The self-assembled collagen fibrils were confirmed by TEM (JEM-1230, JEOL, Tokyo, Japan) after several collagen-coated grids were randomly selected and stained with 1% uranyl acetate for 15 s.

Preparation Dentin Samples for Transmission Electron Microscopy (TEM).

The demineralized or remineralized dentin specimens were fixed in Karnovsky's fixative and postfixed in 1% osmium tetroxide. After fixation, each specimen was rinsed three times with phosphate buffer saline (PBS). The specimens were dehydrated in an ascending series of ethanol (30–100%) and acetone. Then, they were subsequently embedded in epoxy resin. Ultrathin sections (70–90 nm) were prepared

and examined by TEM (JEM-1230, JEOL, Tokyo, Japan) operated at 80 kV.

SUPPLEMENTARY RESULTS AND DISCUSSION

Preparation of a Single-layer Collagen Model. In this study, the TEM image of the negatively stained reconstituted collagen showed a typical banding pattern with a 67 nm periodicity with a characteristic of self-assembled collagen fibrils (Figure S1).¹ The discrete collagen fibrils were approximately 50–150 nm in width (Figure S1).

SUPPLEMENTARY FIGURES

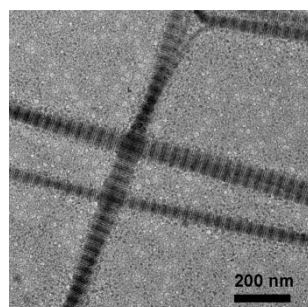


Figure S1. TEM image of the stained self-assembly collagen fibrils shows a typical D-band pattern with the d-spacing about 67 nm.

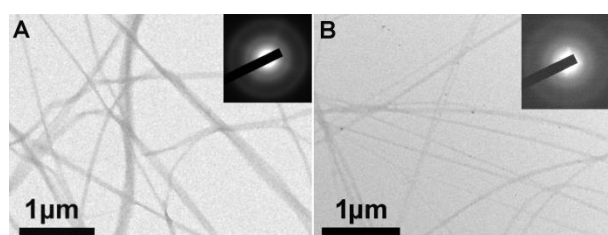


Figure S2. TEM images of the unstained collagen fibrils in control groups ((A) adhesive S3; (B) adhesive AEO) after 14-day incubation. The SAED patterns (insets in A and B) indicate that the collagen fibrils were not mineralized.

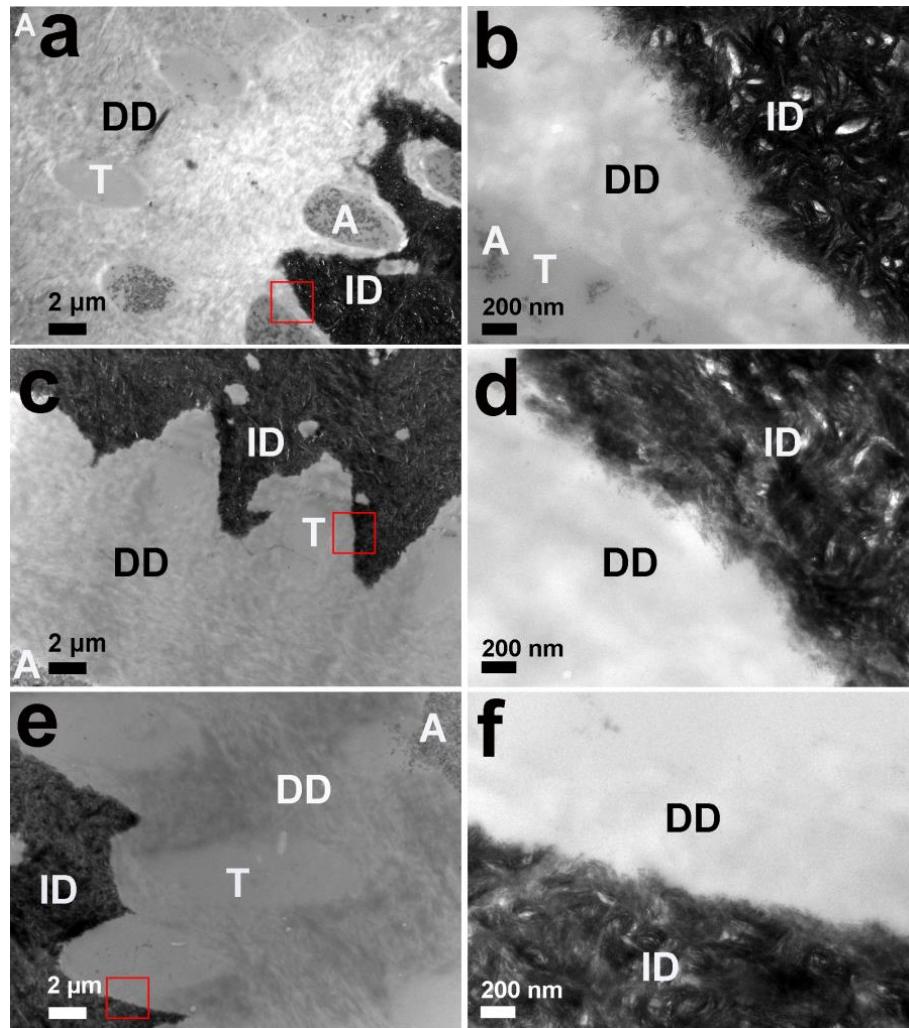


Figure S3. TEM images of dentin specimens in group 1 (adhesive: S3). The remineralization of the demineralized dentin applied with adhesive S3 was not detected after 1-day (panels a-b), 1-month (panels c-d), and 3-month (panels e-f) incubation in artificial saliva. Panels b, d, and f are the higher magnifications of the marked areas (red boxes) in panels a, c, and e, respectively. A: adhesive. R: remineralized dentin. DD: demineralized dentin. ID: intact dentin. T: dentinal tubule.

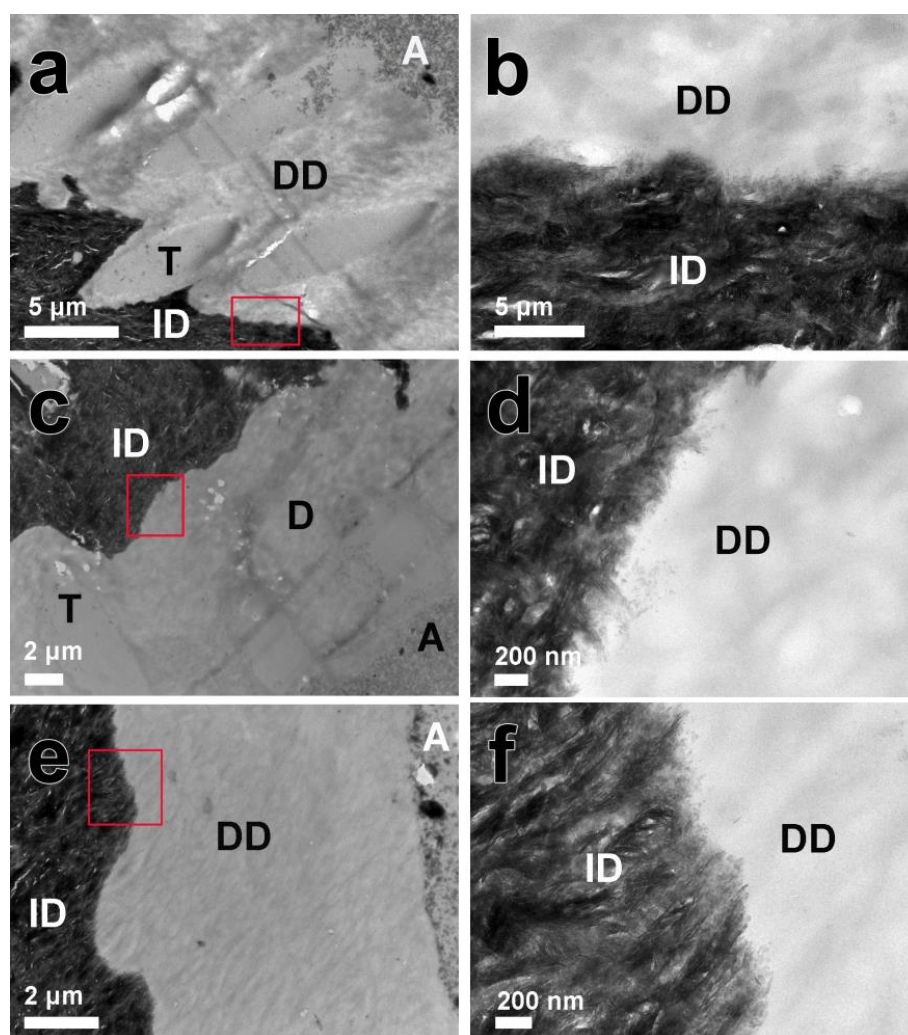


Figure S4. TEM images of dentin specimens in group 2 (adhesive: AEO). The remineralization of the demineralized dentin applied with adhesive AEO was not detected after 1-day (panels a-b), 1-month (panels c-d), and 3-month (panels e-f) incubation in artificial saliva. Panels b, d, and f are the higher magnifications of the marked areas (red boxes) in panels a, c and e, respectively. A: adhesive. R: remineralized dentin. DD: demineralized dentin. ID: intact dentin. T: dentinal tubule.

SUPPLEMENTARY REFERENCES

- (1) Shoulders, M. D.; Raines, R.T. Collagen Structure and Stability. *Annu. Rev. Biochem.* **2009**, 78, 929-958.