Supporting Information

Tannic Acid Induces Dentin Biomineralization by Crosslinking and Surface Modification

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Number of pages:7

Number of figures:4

Number of tables:1

1. Supplementary Experiment Section

1.1 Dentin Collagen Treated by Collagenase

Table S. Test procedure of the HYP assay kit

	Blank group	Standard group	test group
Double distilled water (mL)	1.0		
5μg/ml Standard solution (mL)		1.0	
Test solution (mL)			1.0
Solution 1 (mL)	0.5	0.5	0.5
	Mixing and bathing for 10 min		
Solution 2 (mL)	0.5	0.5	0.5
	Mixing and bathing for 5 min		
Solution 3 (mL)	0.5	0.5	0.5

The samples were water bathed in water at 37°C for 15 minutes and centrifuged at 3500 r/min for 10 minutes. The supernatant was removed, and the absorbance of each sample was measured at a wavelength of 550 nm. The HYP content was calculated by the following formula.

HYP content (μg/ml) = (optical density (OD) value of the test group- OD value of the standard group) / (OD value of the standard group- OD value of the blank group) × concentration of the standard solution (5 μg/ml) × total volume of the sample (mL) / volume of the test sample (mL)

1.2 The Mineralization Degree of Collagen

Since the mineralized part of collagen fibers contain heavy atoms (Ca and P), while the unmineralized part contains relatively light atoms (C, H, and N), the degree of mineralization can be calculated. According to the method of Smeets et al,[1] the pixels of the collagen mineralized parts (S1) and the collagen non-mineralized parts (S2) can be obtained using ImageJ software. At least 5 TEM images were analyzed in each group, and the collagen mineralization rate = S1 ÷ (S1+S2) × 100%.

1.3 Statistical Analysis

All the results presented in this study were the mean values with standard deviations. The data were analyzed statistically using SPSS 24.0 software (IBM SPSS Inc, Chicago, USA), and the significance level was set to 5% ($P < 0.05$). The normality of the data was evaluated by the Kolmogorov-Smirnov test.

2. Supplementary Figures
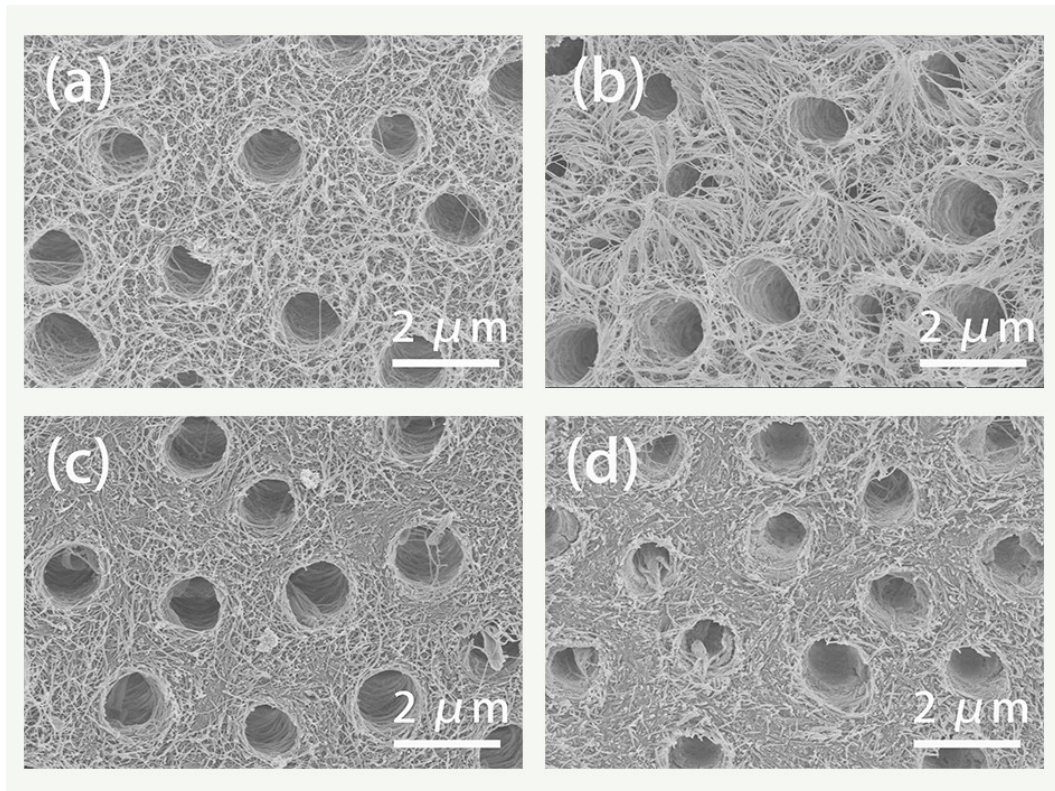
Fig. S1 SEM images illustrating the change in the micromorphology of collagen fibrils after 5 days of exposure to a collagenase solution for the four groups of samples. (a) Demineralized dentin collagen samples; (b) dentin collagen samples with only TA crosslinking; (c) untreated dentin samples with 7 days of remineralization; (d) dentin samples with TA crosslinking and 7 days of remineralization.
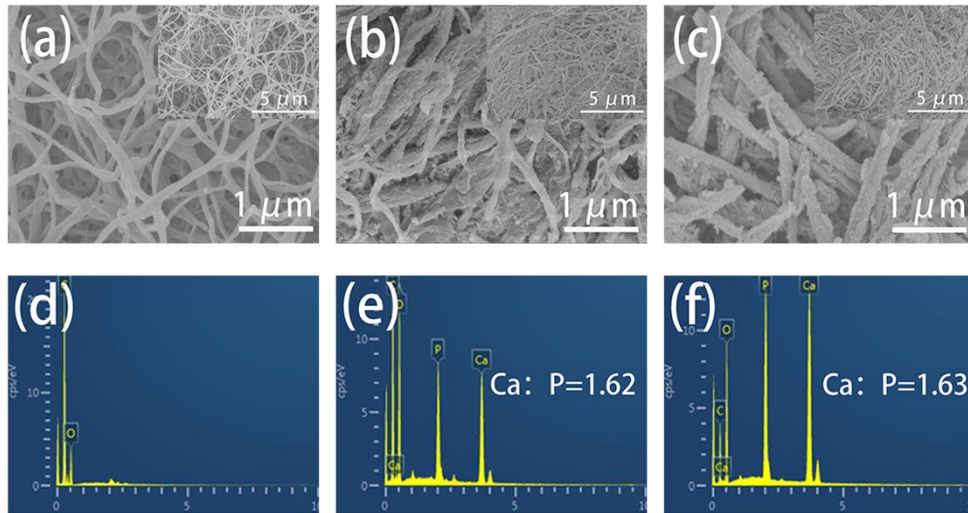
Fig. S2 SEM-EDS images of the surface morphology and element analysis of the collagen membranes for nature (a and d), 10days of mineralization without TA pretreatment (b and e) and 10 days of mineralization with TA pretreatment (c and f). Inset: the low magnitude SEM images.
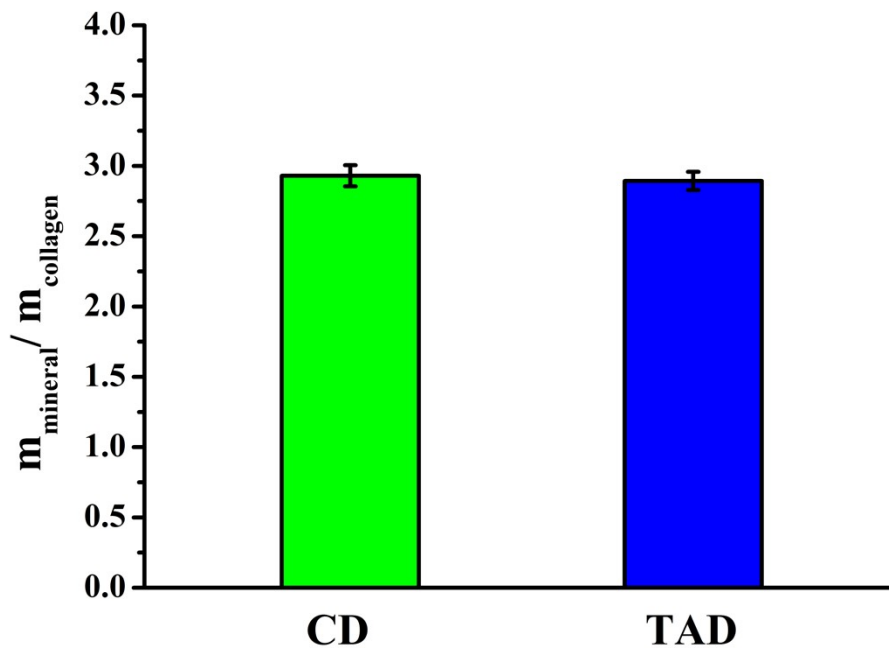
Fig. S3 The mineral contents after demineralization of dentin with EDTA. CD: 4d remineralization of C-dentin, and TAD: 4 days remineralization of TA-dentin. There was no difference between the two groups after statistical analysis ($p > 0.05$).
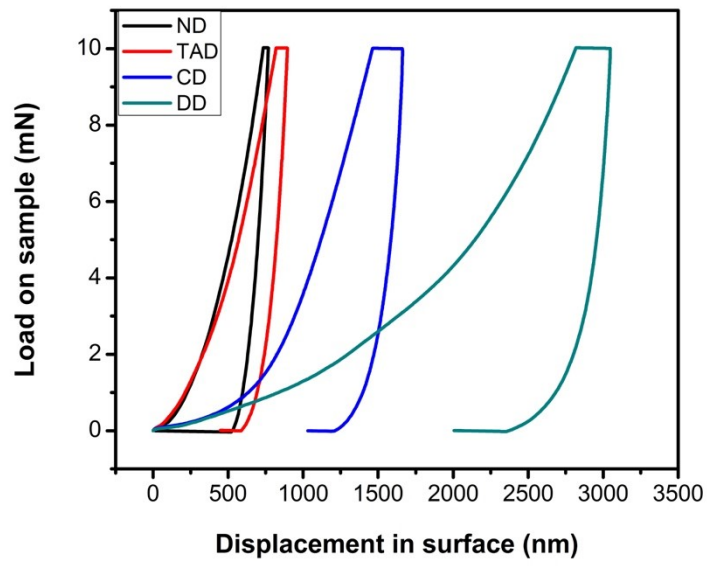
Fig. S4 Load-displacement curve of the dentin. (ND: natural dentin; DD: demineralized dentin, CD: 4d remineralization of C-dentin, and TAD: 4d remineralization of TA-dentin)

References

1. P. J. M. Smeets, A. R. Finney, W. Habraken, F. Nudelman, H. Friedrich, J. Laven, J. J. De Yoreo, P. M. Rodger and N. Sommerdijk, *Proc Natl Acad Sci U S A*, 2017, **114**, E7882-E7890.