Intrafibrillar mineralization of type I collagen with calcium carbonate and strontium carbonate induced by the polyelectrolyte-cation complexes

Supporting Information

Materials and Methods

1. Intrafibrillar mineralization with Sr-doped $CaCO_3$

1.1 Preparation of reconstituted single-layer collagen models.

The reconstituted single-layer collagen models were prepared as those described in section 2.3.1.

1.2 Preparation of Sr-doped pAsp-Ca suspensions.

Three groups were divided based on different doping ratios: 5 mol %, 10 mol % and 15 mol %. The respective amounts of $CaCl_2 \cdot 2H_2O$ were 19 g, 18g and 17 g, and those of $SrCl_2 \cdot 6H_2O$ were 1.814 g, 3.627 g and 5.441 g, respectively. The $CaCl_2 \cdot 2H_2O$/ $SrCl_2 \cdot 6H_2O$ mixtures were dissolved in 10 mL of 10 g/L pAsp solution. The pH value was adjusted to 9.5 ± 0.2 with 10 M NaOH.

1.3 Mineralization of single-layer type I collagen fibrils and characterization.

The reconstituted type I collagen fibrils were treated with the carbonate-bicarbonate buffer for 3 h, and then in Sr-doped pAsp-Ca suspension for 30 min. The grids were gently rinsed with deionized water and dehydrated in an ascending series of ethanol (50-100 v/v %). The mineralized collagen fibrils were finally evaluated with TEM,

HRTEM with SAED and EDX.

2. Spatial distribution of the pAsp-Ca complexes within the collagen fibrils

The laser confocal culture dishes assembled with collagen fibrils were prepared and the assembled collagen fibrils were immunofluorescently labeled with rabbit anti-collagen I antibody and goat anti-rabbit Alexafluor 647 antibody as those described in section 2.3.3. The FITC-pAsp-Ca suspension was prepared as the protocols described in our published article.[1] The assembled collagen fibrils were treated with carbonate-bicarbonate buffer for 3 h, and then FITC-pAsp-Ca suspension for 30 min. The collagen fibrils were observed using a Nikon Ti-E inverted microscope (Nikon, Japan). The 3D-STORM images and videos were examined using Nikon NIS-Elements AR 4.51 software.

Results

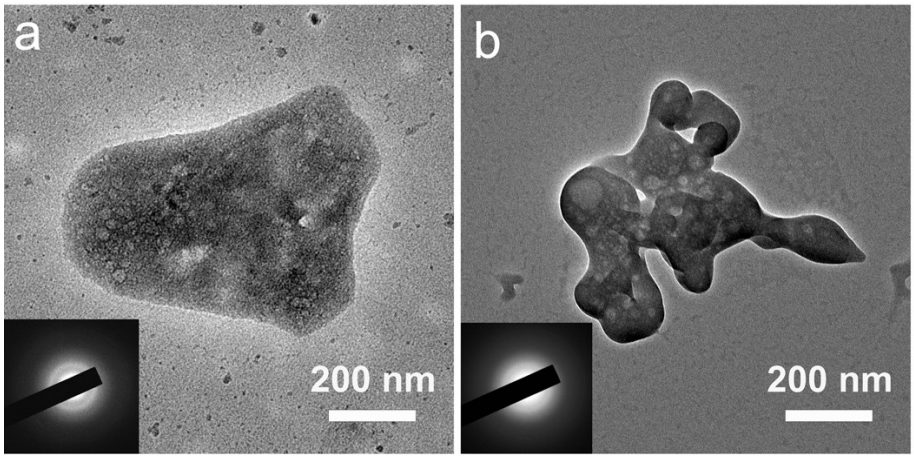


Fig. S1 (a-b) HRTEM images with SAED patterns of the pAsp-Ca complex (a) and pAsp-Sr complex (b). The SAED patterns inset both reveal an amorphous phase.

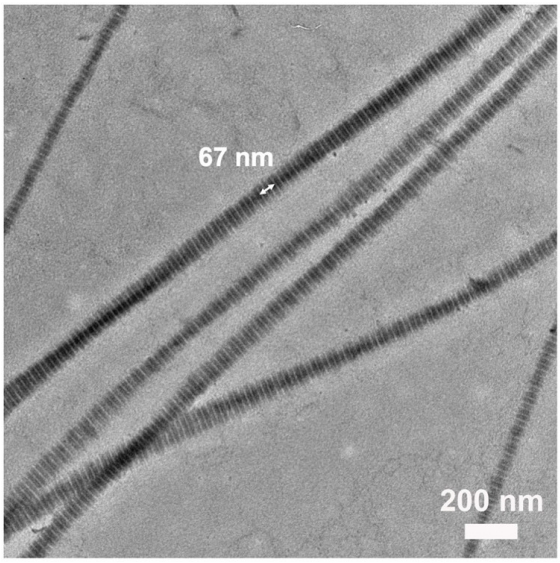
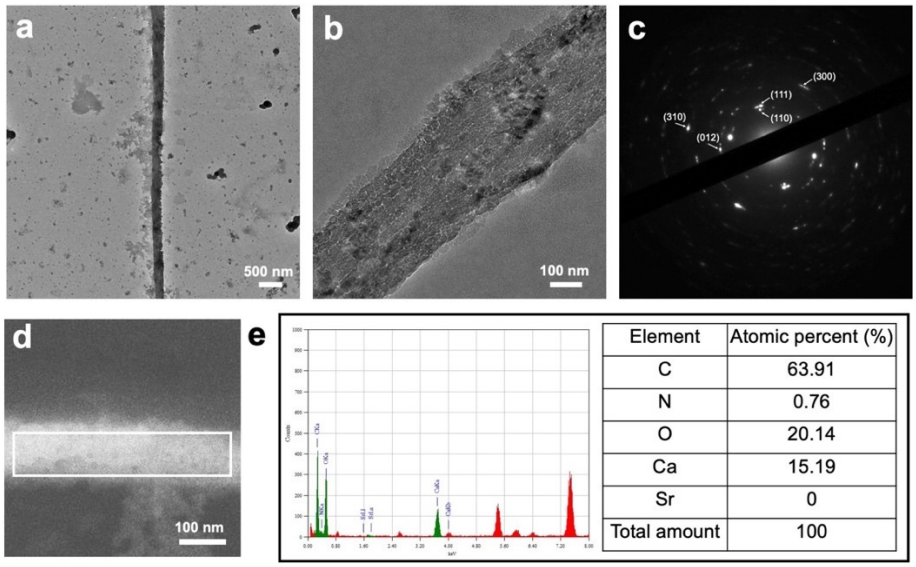


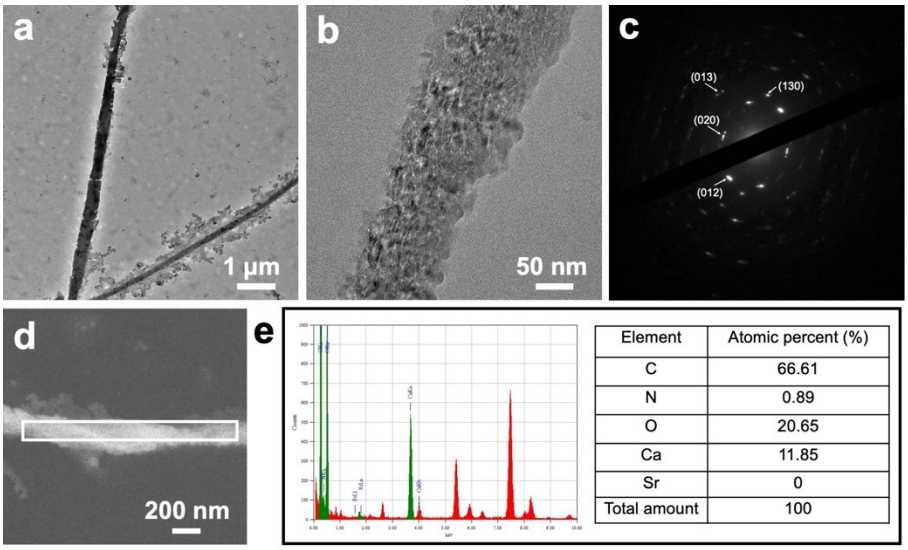
Fig. S2 TEM image of the reconstituted single-layer type I collagen fibrils stained with uranyl acetate.



Element	Atomic percent (%)
C	63.91
N	0.76
O	20.14
Ca	15.19
Sr	0
Total amount	100

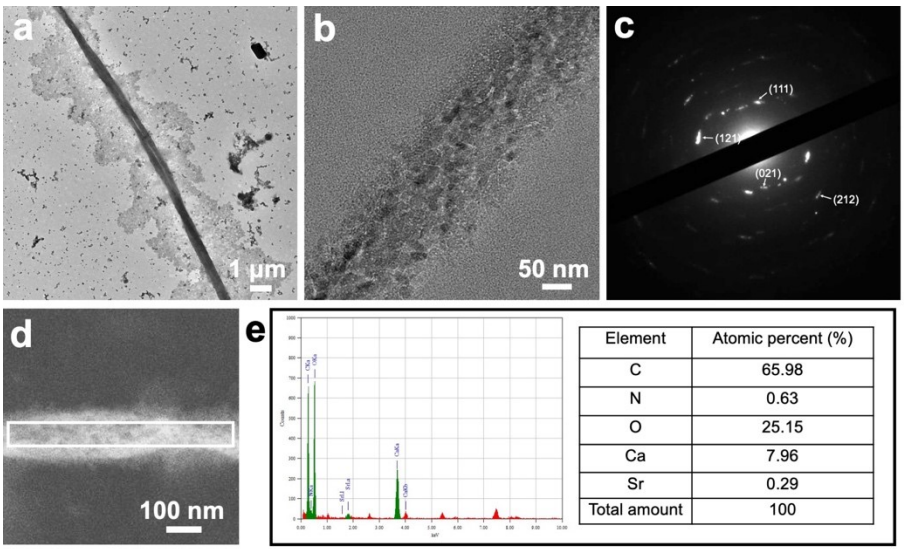
Fig S3 TEM, HRTEM with SAED and EDX results of the collagen fibrils mineralized with Sr-doped $CaCO_3$ (doping ratio: 5 mol%). (a) TEM image of a mineralized collagen fibril. (b) HRTEM image of a mineralized collagen fibril shows the deposition of intrafibrillar crystallites. (c) SAED pattern of the collagen fibril in “b”. Aragonite (PDF 33-0268) is represented by (111), (012) and (310) planes, whereas the vaterite (PDF

41-1475) is represented by (110) and (300) planes. (d) The darkfield image of a mineralized collagen fibril. The area in the white box was analyzed by the EDX. (e) EDX results indicate that no Sr element was detected in the test zone.



Element	Atomic percent (%)
C	66.61
N	0.89
O	20.65
Ca	11.85
Sr	0
Total amount	100

Fig. S4 TEM, HRTEM with SAED and EDX results of the collagen fibrils mineralized with Sr-doped $CaCO_3$ (doping ratio: 10 mol%). (a) TEM image of mineralized collagen fibrils. (b) HRTEM image of a mineralized collagen fibril shows the intrafibrillar crystallites. (c) SAED pattern of the collagen fibril in “b”. Aragonite (PDF 33-0268) is represented by (020), (012), (013) and (130) planes. (d) The darkfield image of a mineralized collagen fibril. The area in the white box was analyzed by the EDX. (e) No Sr element was detected in the test zone according to the EDX results.



Element	Atomic percent (%)
C	65.98
N	0.63
O	25.15
Ca	7.96
Sr	0.29
Total amount	100

Fig. S5 TEM, HRTEM with SAED and EDX results of the collagen fibrils mineralized with Sr-doped $CaCO_3$ (doping ratio: 15 mol%). (a) TEM image of the mineralized collagen fibrils. (b) HRTEM image of a mineralized collagen fibril shows the deposition of intrafibrillar crystallites. (c) SAED pattern of the collagen fibril in "b". Aragonite (PDF 33-0268) is represented by (021), (111), (121) and (212) planes. (d) The darkfield image of a mineralized collagen fibril. The area in the white box was analyzed by the EDX. (d) The EDX results show that the incorporation ratio of Sr was 3.5 mol% (Sr / (Ca + Sr) = 3.5 mol%).

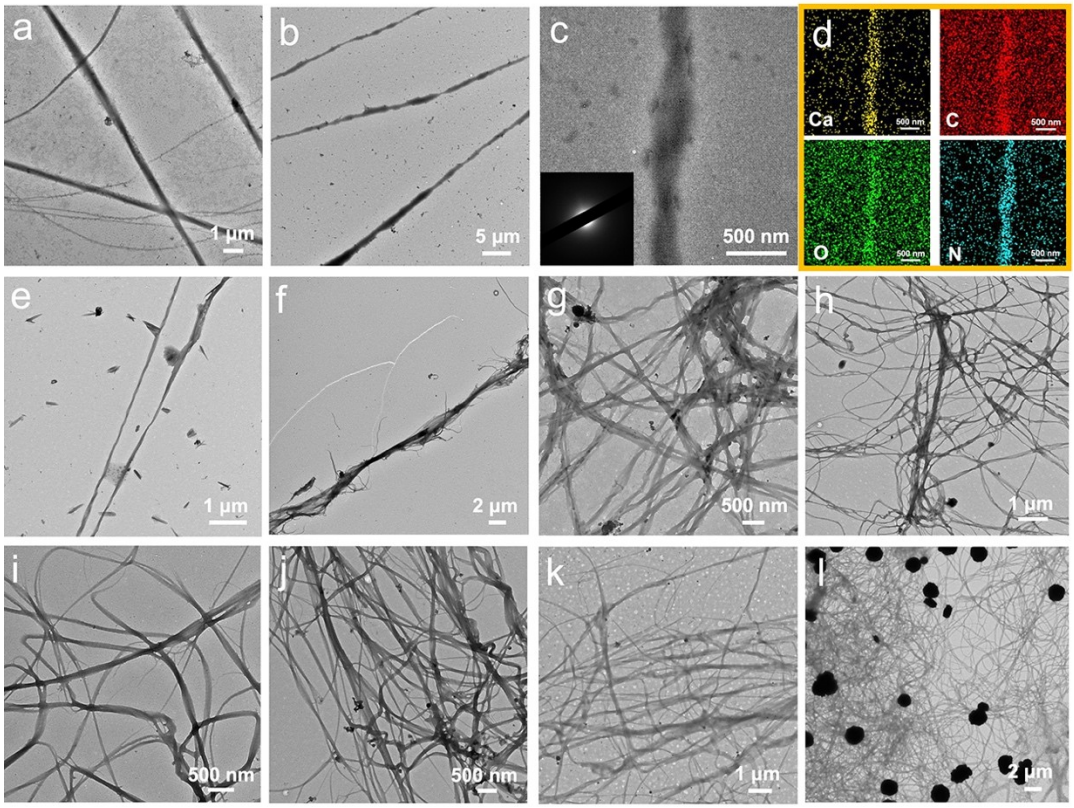


Fig. S6 Characterization of the collagen fibrils treated with the pAsp-Ca suspension followed by the carbonate-bicarbonate buffer. (a) The collagen fibrils became darker after the incubation in the pAsp-Ca suspension for 5 min. (b) The electron density of the collagen fibrils increased after the fibrils were sequentially incubated in the pAsp-Ca suspension for 5 min and carbonate-bicarbonate buffer for 5 min. Amorphous mineral precursors seem to deposit inside the fibrils. (c) HRTEM image with SAED pattern of a collagen fibril that was sequentially incubated in the pAsp-Ca suspension for 5 min and carbonate-bicarbonate buffer for 5 min. The HRTEM image shows an increase in the electron density of the fibril. The SAED pattern inset reveals an amorphous phase of the intrafibrillar minerals. (d) Elemental mapping images of the collagen fibril in "c" (Ca: yellow, C: red, O: green, N: blue). (e-l) No intrafibrillar mineralization was observed after the collagen fibrils were sequentially incubated in

the pAsp-Ca suspension for 5 min and carbonate-bicarbonate buffer for 15 min (e), 30 min (f), 1 h (g), 2 h (h), 3 h (i), 4 h (j), 5 h (k) and 6 h (l).

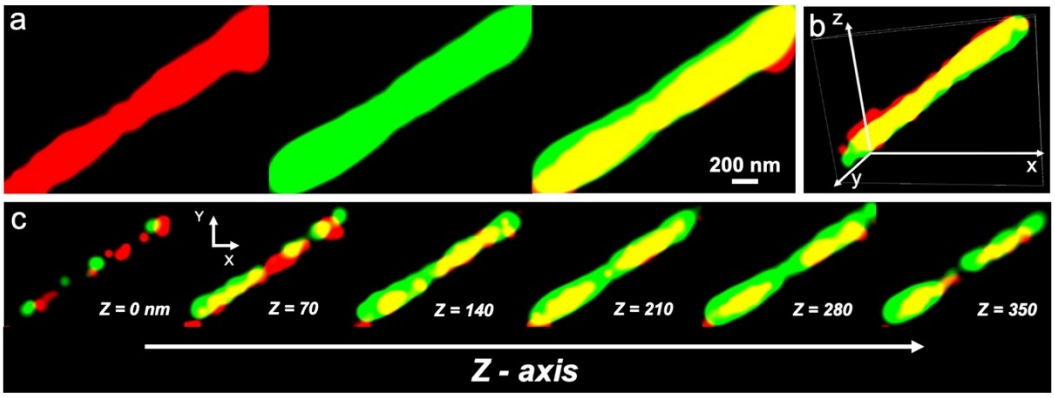


Fig. S7 The STORM images of the collagen fibril after the sequential incubation in carbonate-bicarbonate buffer for 3 h and pAsp-Ca suspension for 30 min. (a) The immunofluorescently labeled collagen fibril emitted red fluorescence. FITC-labeled pAsp molecules emitted green fluorescence. Red fluorescence and green fluorescence were merged in yellow. (b) 3D visualization of the spatial distribution of intrafibrillar pAsp molecules. (c) The z-slices of the STORM images in “a” with an interval of 70 nm.